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NOTE ON a -VALUES OF THE RIEMANN ZETA FUNCTION NEAR THE CRITICAL LINE

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ABSTRACT. We count the number of a -values of the Riemann zeta function near the critical line. Previously an asymptotic formula for the number of a -values in $\text{Re}(s) > \frac{1}{2} + \frac{1}{(\log T)^\theta}$ and $T < \text{Im}(s) < 2T$ for $0 < \theta < \frac{1}{13}$ is known. We improve it by proving the same asymptotic formula for $0 < \theta < \frac{1}{7}$.

1. INTRODUCTION

The Riemann zeta function is defined by the Dirichlet series

$$\zeta(s) = \sum_{n=1}^{\infty} \frac{1}{n^s}$$

for $\text{Re}(s) > 1$ and its analytic continuation to $\mathbb{C}$ except for a simple pole at $s = 1$. It has an Euler product expression

$$\zeta(s) = \prod_p \left(1 - \frac{1}{p^s}\right)^{-1}$$

for $\text{Re}(s) > 1$, where p runs over the prime numbers. By finding a zero free region of $\zeta(s)$ in the critical strip near $\text{Re}(s) = 1$, the prime number theorem was first proved in 1896, which says that the number of primes $\leq x$ is asymptotically $\int_2^x \frac{dt}{\log t}$. More precise estimates are equivalent to bigger zero free regions of $\zeta(s)$ and the largest possible zero free region corresponds to the Riemann Hypothesis, say $\zeta(s)$ is nonvanishing for $\text{Re}(s) > \frac{1}{2}$. See [13] for the details. This motivates to construct various zeta functions and L -functions, and they become major research topics in analytic number theory.

Let a be a nonzero complex number, then we call $\rho_a = \beta_a + i\gamma_a$ as an a -value of $\zeta(s)$ if $\zeta(\rho_a) = a$. By the Dirichlet series of $\zeta(s)$, there exists a constant A depending on a such that $\zeta(s) \neq a$ for $\text{Re}(s) > A$. Essentially by the functional equation of $\zeta(s)$, it is known that there are (trivial) a -values near $s = -2, -4, -6, \dots$ and at most finitely many more a -values in $\text{Re}(s) < 0$. All the other (nontrivial) a -values are in the strip $0 \leq \text{Re}(s) \leq A$ and they are infinitely many. Moreover, for any fixed $\frac{1}{2} < \sigma_1 < \sigma_2$, the number of a -values with $\sigma_1 < \beta_a < \sigma_2$ and $T < \gamma_a < 2T$ is asymptotically $c(\sigma_1, \sigma_2)T$ as $T \rightarrow \infty$. Here, the constant $c(\sigma_1, \sigma_2) \geq 0$ has an integral representation in terms of the Jensen function and it is positive if $\frac{1}{2} < \sigma_1 \leq 1$. See [1] for a proof.

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2. MAIN RESULTS

In this short note, we count the number of a -values near the critical line. Let

$$\sigma_T := \sigma_T(\theta) = \frac{1}{2} + \frac{1}{(\log T)^\theta}$$

for $\theta > 0$ and define $N_a(\sigma; T_1, T_2)$ by the number of a -values of $\zeta(s)$ with $\beta_a > \sigma$ and $T_1 < \gamma_a < T_2$. Then the following main theorem improves [2, Theorem 1.1].

Theorem 2.1. *Let $0 < \theta < \frac{1}{7}$ be fixed, then we have*

$$N_a(\sigma_T; T, 2T) = \frac{T(\log T)^\theta}{8\pi^{3/2}\sqrt{\theta \log \log T}} + O\left(\frac{T(\log T)^\theta}{(\log \log T)^{3/4}}\right)$$

as $T \rightarrow \infty$.

Theorem 2.1 is a consequence of the following theorem by a standard application of Littlewood's lemma. For a proof of the deduction, see [2, p. 861–862].

Theorem 2.2. *Let $0 < \theta < \frac{1}{7}$ be fixed, then we have*

$$\frac{1}{T} \int_T^{2T} \log |\zeta(\sigma_T + it) - a| dt = \frac{\sqrt{\psi_T}}{2\sqrt{\pi}} + \frac{\log |a|}{2} + \frac{(\log |a|)^2}{2\sqrt{\pi}\psi_T} + O\left(\frac{1}{\log \log T}\right)$$

as $T \rightarrow \infty$, where $\psi_T := \sum_p \sum_{k \geq 1} \frac{1}{k^2 p^{2k\sigma_T}}$.

Note that $\psi_T = \theta \log \log T + O(1)$ by the prime number theorem.

To prove Theorem 2.2, we approximate the distribution of the Riemann zeta function in the right half of the critical strip by the distribution of its probabilistic model, so called the random zeta function. It is defined by the product

$$\zeta(\sigma, \mathbb{X}) := \prod_p \left(1 - \frac{\mathbb{X}(p)}{p^\sigma}\right)^{-1}, \quad (2.1)$$

which is convergent almost surely for $\sigma > \frac{1}{2}$. Let

$$\Phi_{\sigma, T}(\mathcal{B}) := \frac{1}{T} \text{meas}\{t \in [T, 2T] : \log \zeta(\sigma + it) \in \mathcal{B}\}$$

and

$$\Phi_\sigma^{\text{rand}}(\mathcal{B}) := \mathbb{P}(\log \zeta(\sigma, \mathbb{X}) \in \mathcal{B})$$

for a Borel set $\mathcal{B}$ in $\mathbb{C}$. Then we define the discrepancy between the two measures by

$$\mathbf{D}_T(\sigma) := \sup_{\mathcal{R}} |\Phi_{\sigma, T}(\mathcal{R}) - \Phi_\sigma^{\text{rand}}(\mathcal{R})|,$$

where $\mathcal{R}$ runs over all rectangular boxes in $\mathbb{C}$ with sides parallel to the coordinate axes and possibly unbounded. There are several results which show that the discrepancy $\mathbf{D}_T(\sigma)$ is small for fixed $\frac{1}{2} < \sigma < 1$ in [3], [5], [10–12] and for σ near $\frac{1}{2}$ in [2], [4], [7–9]. The latest one is the following theorem.

Theorem 2.3 (Theorem 1.1 in [9]). *Assume that $\log \log T \leq G(T) \leq \frac{\log T}{(\log \log T)^2}$, then we have*

$$\mathbf{D}_T\left(\frac{1}{2} + \frac{1}{G(T)}\right) \ll \frac{\sqrt{G(T)} \log \log T}{\sqrt{\log T}}$$

as $T \rightarrow \infty$.

By applying Theorem 2.3 to the arguments in [2, Section 4] we will prove Theorem 2.2.

Proof of Theorem 2.2. The core of the proof is that we can choose

$$L = (\log T)^{\frac{1}{2}(1-\theta)}, \quad (2.2)$$

which is bigger than L in [2]. Let A be a large fixed constant and

$$\begin{aligned} \mathcal{R}_1 &:= [\log |a| + 1/L, A \log \log T] \times [-A \log \log T, A \log \log T], \\ \mathcal{R}_2 &:= [-A \log \log T, \log |a| - 1/L] \times [-A \log \log T, A \log \log T], \\ \mathcal{R}_3 &:= (\log |a| - 1/L, \log |a| + 1/L) \times [-A \log \log T, A \log \log T] \end{aligned}$$

and

$$\mathcal{S}_j = \{t \in [T, 2T] : \log \zeta(\sigma_T + it) \in \mathcal{R}_j\}$$

for $j = 1, 2, 3$. By [2, p.857] we find that

$$\frac{1}{T} \int_T^{2T} \log |\zeta(\sigma_T + it) - a| dt = \sum_{j=1,2,3} \frac{1}{T} \int_{\mathcal{S}_j} \log |\zeta(\sigma_T + it) - a| dt + O\left(\frac{(\log \log T)^2}{(\log T)^{5-3\theta}}\right).$$

Next, we apply the discrepancy result to the last integrals. By Theorem 2.3 and the same method as in [6, p. 486–489] we have

$$\begin{aligned} \frac{1}{T} \int_{\mathcal{S}_j} \log |\zeta(\sigma_T + it) - a| dt &= \int_{\mathcal{R}_j} \log |e^{u+iv} - a| d\Phi_{\sigma_T, T}(u, v) \\ &= \int_{\mathcal{R}_j} \log |e^{u+iv} - a| d\Phi_{\sigma_T}^{\text{rand}}(u, v) + O\left(\frac{(\log \log T)^3}{L}\right) \end{aligned}$$

for $j = 1, 2$. We cannot do the same for $j = 3$ since the integrand has logarithmic singularities in $\mathcal{R}_3$. Instead we apply a high moment estimate [2, Lemma 4.1]. By Theorem 2.3 we have

$$\frac{1}{T} |\mathcal{S}_3| = \Phi_{\sigma_T, T}(\mathcal{R}_3) = \Phi_{\sigma_T}^{\text{rand}}(\mathcal{R}_3) + O\left(\frac{\log \log T}{L}\right).$$

By [2, Proposition 3.1] and [5, Lemma 7.2] we find that

$$\Phi_{\sigma_T}^{\text{rand}}(\mathcal{R}_3) \ll \int_{\log |a| - 1/L}^{\log |a| + 1/L} \int_{-A \log \log T}^{A \log \log T} e^{-\frac{\lambda}{\theta \log \log T}(u^2 + v^2)} dv du \ll \frac{\sqrt{\log \log T}}{L}$$

for some constant $\lambda > 0$. Thus,

$$\frac{1}{T} |\mathcal{S}_3| \ll \frac{\log \log T}{L}.$$

By Hölder's inequality and [2, Lemma 4.1] with $k = \log \log T$ we have

$$\begin{aligned} \left| \frac{1}{T} \int_{\mathcal{S}_3} \log |\zeta(\sigma_T + it) - a| dt \right| &\leq \left(\frac{1}{T} |\mathcal{S}_3| \right)^{1 - \frac{1}{2k}} \left(\frac{1}{T} \int_T^{2T} (\log |\zeta(\sigma_T + it) - a|)^{2k} dt \right)^{\frac{1}{2k}} \\ &\ll \frac{(\log \log T)^2}{(\log T)^{\frac{1}{2}(1-7\theta)}}. \end{aligned}$$

Hence, we obtain

$$\frac{1}{T} \int_T^{2T} \log |\zeta(\sigma_T + it) - a| dt = \sum_{j=1,2} \int_{\mathcal{R}_j} \log |e^{u+iv} - a| d\Phi_{\sigma_T}^{\text{rand}}(u, v) + O\left(\frac{(\log \log T)^2}{(\log T)^{\frac{1}{2}(1-7\theta)}}\right). \quad (2.3)$$

Finally, by [2, Section 4] we find that

$$\sum_{j=1,2} \int_{\mathcal{R}_j} \log |e^{u+iv} - a| d\Phi_{\sigma_T}^{\text{rand}}(u, v) = \frac{\sqrt{\psi_T}}{2\sqrt{\pi}} + \frac{\log |a|}{2} + \frac{(\log |a|)^2}{2\sqrt{\pi}\psi_T} + O\left(\frac{1}{\log \log T}\right).$$

Note that the O -term in (2.3) is smaller than $\frac{1}{\log \log T}$ since $\theta < \frac{1}{7}$ is fixed. By combining the above two equations, Theorem 2.2 holds. $\square$

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라만 분광법을 통한 $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ 박막의 포논 분석

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$\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$ 와 같은 구리산화물 고온 초전도체는 불순물의 정도에 의하여 전자구조에 영향을 미친다. 그렇기에 성장과정에서 분자 빔 에피택시 혹은 펄스 레이저 증착과 같은 방식으로 제작되는 박막은 순도를 높일 수 있어 초전도 연구에 있어 중요하게 사용된다. 그러나 박막의 얇은 두께와 기판의 영향으로 인하여 라만 분광학을 이용한 연구는 강상관계물질 에서의 높은 활용성에도 불구하고 이뤄지지 못하고 있다. 본 연구에서는 이러한 한계를 극복하기 위해 라만 시스템을 활용하고 데이터를 분석방식의 변화를 주어 박막샘플에서 기판의 데이터를 분리하였다. 그 결과 최적 도핑에서 기존에 라만을 통해 연구되었던 벌크 $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ 와는 다르게 225cm^{-1} 근방의 포논이 두개로 갈라지지 않고, 하나로 나타나는 현상을 발견하였다. 이는 샘플 내에서 반전 대칭의 깨짐의 정도가 감소했음을 말한다. 본 연구는 박막샘플에 대한 라만 분광법 연구의 가능성을 말하며, 이를 통하여 시료의 결정성을 파악할 수 있는 접근법임을 제안한다.

Keywords: $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$, 구리산화물 고온 초전도체, 박막, 라만 분광학

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Phonon Analysis of $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ Thin Films via Raman Spectroscopy

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Copper oxide high-temperature superconductors, such as $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$, are influenced by the degree of impurities in their electronic structure. Therefore, thin films produced through methods like molecular beam epitaxy or pulsed laser deposition can enhance purity and are crucial for superconductivity research. However, due to the [thickness](#) of the films and the influence of the substrate, studies using Raman spectroscopy have not been conducted despite its high applicability in strongly correlated materials. In this study, we overcame these limitations by utilizing a Raman system and modifying the data analysis method to separate substrate data from thin film samples. As a result, we discovered that, at optimal doping, phonons around 225 cm^{-1} do not split into two, as previously observed in bulk $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ studied via Raman spectroscopy, but instead appear as a single peak. This indicates a reduction in the degree of broken inversion symmetry within the sample. This research highlights the potential of Raman spectroscopy studies on thin film samples and proposes an approach to assess the crystallinity of the samples.

Keywords: $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$, cuprate high temperature superconductors, [thin film](#), Raman spectroscopy

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1. 서론

1986 년 J.G. 베드노르즈와 K.A. 필리에 의하여 약 30 K 이상의 임계온도를 가지는 페로브스카이트 구조를 지닌 구리산화물 기반 고온 초전도체인 $\text{Ba}_x\text{La}_{5-x}\text{Cu}_5\text{O}_{5(3-y)}$ 가 발견되었다. 이러한 발견은 최초로 기존 BCS 이론의 상한선인 약 30 K 이상에서 초전도성을 갖는 고온 초전도 물질이었다[1]. 그렇기에 기존 BCS 이론 만으로는 고온 초전도 현상의 근본 원리에 대하여 설명하지 못하였으며, 이를 설명하기 위해 다양한 연구들이 진행되었다[2-4]. 또한 연구가 진행됨에 따라 많은 구리산화물 기반 고온 초전도체를 발견하였으며, 이러한 발견은 더 높은 임계온도를 찾아 기술적으로 응용가능성을 활용할 수 있는 기회를 기대하게 되었다.

이러한 구리산화물 초전도체에서 가장 중요하게 다뤄지는 영역은 CuO_2 평면이다[5]. 초전도 상태는 정상상태보다 더 낮은 에너지를 갖는다[6]. 초전도 현상이 일어나게 되면 쿠퍼쌍이 생성되고, 이러한 쿠퍼쌍에 의해서 전류가 흐르면 더욱 낮은 에너지를 가지기 때문에[6] 초전도 연구에서 중요하게 여기는 영역이다. 그리고 구리산화물 고온 초전도체에서 이러한 쿠퍼쌍이 생기는 영역이 CuO_2 평면이기에 구리산화물 초전도체를 연구함에 있어 중요하게 다뤄지고 있다[4]. 본 연구에서 사용된 $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$ (LSCO)는 기본 단위 셀에 CuO_2 단일층을 가지고 있어, $\text{YBa}_2\text{Cu}_3\text{O}_y$ 처럼 사슬에 의한 현상이나, $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+x}$ 처럼 구조적으로 뒤틀림과 관련된 부분을 고려하지 않아도 된다. 이 때문에 LSCO 는 단순히 Sr 의 도핑 농도에 따라서 홀 도핑 농도가 결정되며, 도핑 농도에 의존하는 구리산화물 초전도체의 특성을 연구하는 데에 매우 적합하다 [7, 8].

또한 구리산화물 초전도체는 격자, 스핀, 전자 등이 강하게 상호작용 되어 그로 인한 상이 보이기 때문에 강하게 상호작용 된 전자 시스템에 속해 있다. 그렇기에 이를 연구하기 위해 동시에 접근할 수 있는 시스템이 필요하다[7, 9]. 라만 분광학은 직간접적으로 포논, 전자, 마그논

등 준입자에 동시에 접근할 수 있는 유용한 도구이므로 고온 초전도를 연구함에 있어 사용되고 있다[10, 11].

그러나 고온 초전도체는 강하게 상호작용 된 전자시스템에 속해 있어, 불순물이나 이상적으로 성장되지 못한 영역에 대하여 더욱 크게 영향을 받는다. 이에 따라 전자적인 구조 혹은 자기적 성질에 대해서도 영향을 받게 되고, 더 나아가서는 초전도 현상에도 영향을 미치게 된다[12, 13].

이 때문에 고온 초전도를 연구함에 있어 시료를 어떻게 성장을 시키는가에 대한 연구 또한 활발하게 이뤄지고 있는데, 그 중에서 가장 시료를 깨끗하고 품질이 좋게 성장시키는 방식이 바로 펄스 레이저 증착(Pulsed Laser Deposition; PLD)과 분자 빔 에피택시(Molecular Beam Epitaxy; MBE)등이 있다[14, 15]. 이러한 방식들은 시료들을 고품질로 기를 수 있으며, 그에 따라 시료의 결정성이 뛰어난 박막을 제작할 수 있다. 이 때문에 고온 초전도 분야에서 이러한 방식들을 통해 기른 박막 시료들을 통하여 활발히 연구되고 있다[16-18].

이러한 박막 시료는 단순히 더 좋은 데이터 뿐이 아닌 다른 성질을 가지기도 한다. 박막 시료와 벌크 시료는 같은 $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$ 라는 분자구조 임에도 불구하고 과도핑 상태에서 도핑 농도가 증가함에 따라 T_c 바로 위의 온도에서 직류 전기전도도 값에 대한 그래프가 서로 다른 양상을 지니고 있다. 박막 시료는 도핑 농도와, 직류 전기전도도 값이 비례하는 반면, 벌크 시료의 경우에는 비례하지 않는다. 이는 박막 시료와 벌크 시료 간의 전기적 성질이 다를 수 있음을 말한다[18].

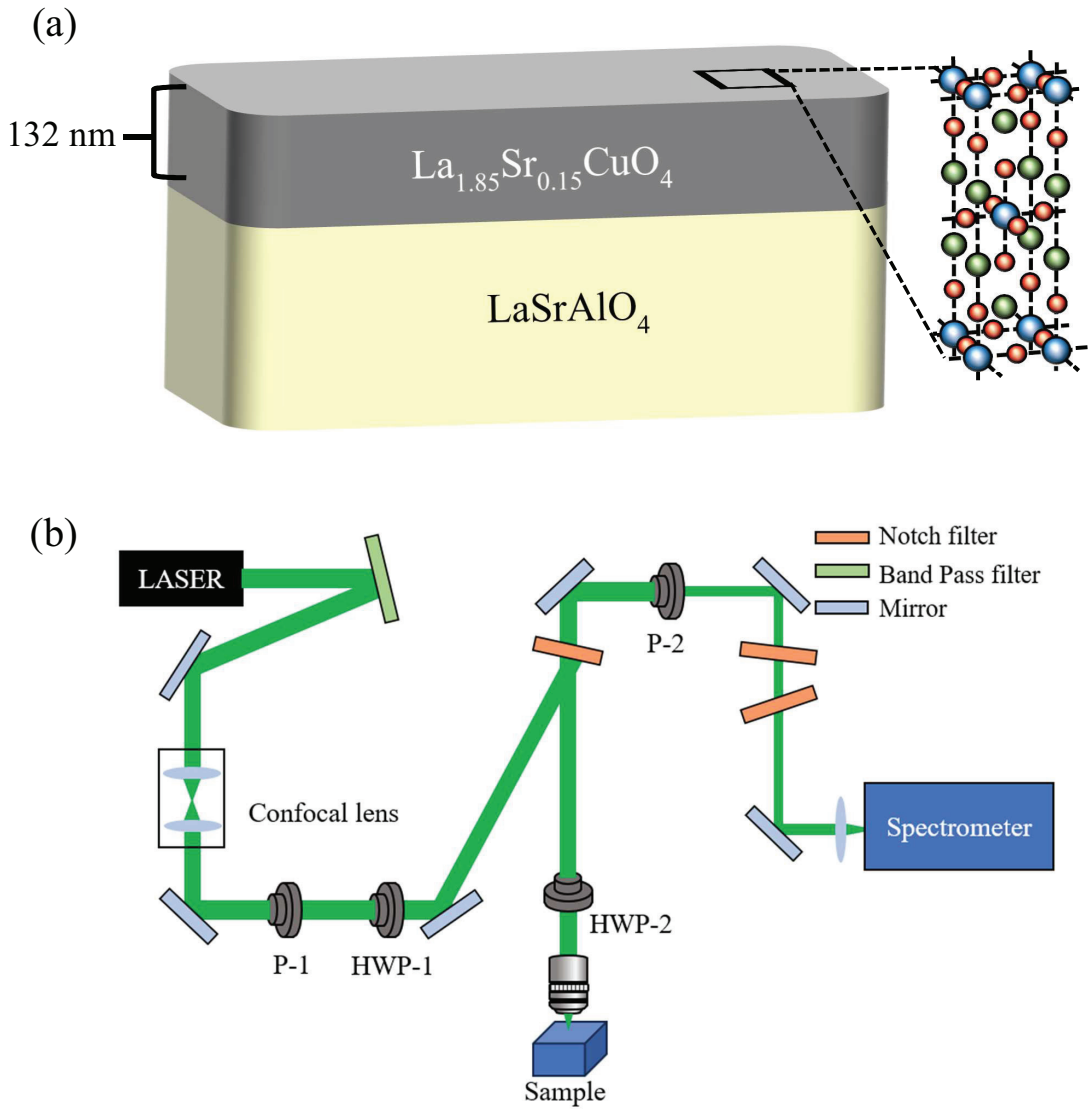
그에 따라 위의 언급한 라만 분광학을 통하여 고온 초전도체 더욱이 나아가서 강상관계 물질을 박막으로 기른 시료들을 연구하는 것 또한 중요하다. 그러나 박막의 특성상 얇은 두께로 인하여 기관의 데이터에 의해 박막의 데이터가 가려지거나, 라만 강도가 약해져 우리가 바라는 박막만의 데이터를 추출하기 힘들다. 그렇기에 현재까지는 박막을 라만을 통해 연구하는 것은 라만의 유용함과 박막 시료의 좋은 결정성과, 다른 물리적 특성에도 불구하고 잘 이뤄지지 않고 있었다.

본 연구에서는 지금까지 연구되지 않았던 LaSrAlO_4 (LSAO) 기판 위에 펄스 레이저 증착으로 100 단위 격자가량 성장시킨 LSCO를 온도 의존성 라만 분광법을 이용하여 분석을 진행하였다. 기존에 제작된 LSCO와 같은 경우는 일반적으로 플러팅 존 방식 혹은 플렉스 방식을 사용하지만, 두 경우 앞서 이야기했듯, 모두 결정성이 좋지 못한 영역이 있거나 불순물이 있다는 결점이 있다. 그렇기에 일반적으로 표면을 깔끔하게 만드는 방식으로 클리브 방식을 사용하지만, LSCO는 유닛셀의 구조상 클리브를 진행할 때 깔끔한 표면을 가지기가 어렵기에 표면을 연마하는 방식을 사용하기도 한다. 그러나 이 방식 역시도 표면이나 내부 구조에 변형을 줄 수 있기에 표면이 중요한 라만 분광학에서의 데이터에 변화를 줄 수 있다. 기존 선행연구된 자료에서 같은 공법으로 제작되었으나 표면을 처리한 방식만의 차이로 라만 데이터의 변화가 있었다. 즉 LSCO의 제작 과정에 따른 라만 데이터에 영향을 줄 수 있다는 것이고, 필름 LSCO는 앞선 플로팅 존 메소드로 제작된 시료에 비하여 깔끔한 표면과 좋은 결정성을 지녀 라만 데이터의 변화를 관측하고자 하였다. 그러나 앞서 언급한 박막의 라만 측정이 힘들기에 박막 시료를 측정하기 위해 실험 과정에 렌즈의 z 높이에 대하여 세밀하게 조정하였고, 분석과정에서 기판의 데이터 분리하는 과정을 거쳤다.

2. 본론

2-1. 실험 과정

시료는 LSAO 기판 위에 펄스 레이저 증착 방식으로 100 유닛셀가량 성장시킨 $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ 를 사용하여 온도 의존성 라만 분광 실험을 진행하였다. 레이저는 532 nm의 파장을 가지는 코볼트사의 다이오드펌프 레이저를 사용하여 파워는 0.5 mW로 유지하면서 측정하였다. 또한 40 배율의 대물렌즈를 사용하였으며, 샘플의 위에 노치필터를 두었다. 이를 통해 입사하는 빛은 532 nm 파장의 빛만이 입사되었고, 산란되어 측정하는 빛의 레일리 산란은 걸러주었다. 이 후 2 개의 노치필터를 사용하여 완전히 걸러지지 못한 레일리 산란을 걸러준다. 이에 $\sim 10 \text{ cm}^{-1}$ 의 저 에너지 영역까지 관측 가능하다. 분광기는 호리바사의 iHR 320을 이용하였고, 1 mm 당 600 개의 슬릿이 있는 회절격자를 사용하여 3.74 cm^{-1} 간격의 라만 시그널을 얻었다. B_{1g} , B_{2g} 채널의 라만 시그널을 얻기 위하여 그림 1-(b)의 P-1(편광판)을 통해 먼저 빛을 한 방향으로 편광 시킨 후 HWP-1(반파장판)에서 나오는 빛과 P-2(편광판)의 편광 방향을 수직하게 만들어 입사광과 산란광을 서로 수직한 방향의 데이터를 얻었다. 이후 샘플을 돌리는 대신 샘플 위에 HWP-2(반파장판)을 돌려 각각 B_{1g} 와 B_{2g} 데이터를 얻었다. 실험을 진행하며 박막시료를 측정하기 위해 렌즈의 높이에 따른 라만 데이터의 변화를 측정한 후 기판의 포논 피크가 가장 적게 나타나는 높이로 렌즈의 높이를 세밀하게 조정하였다.

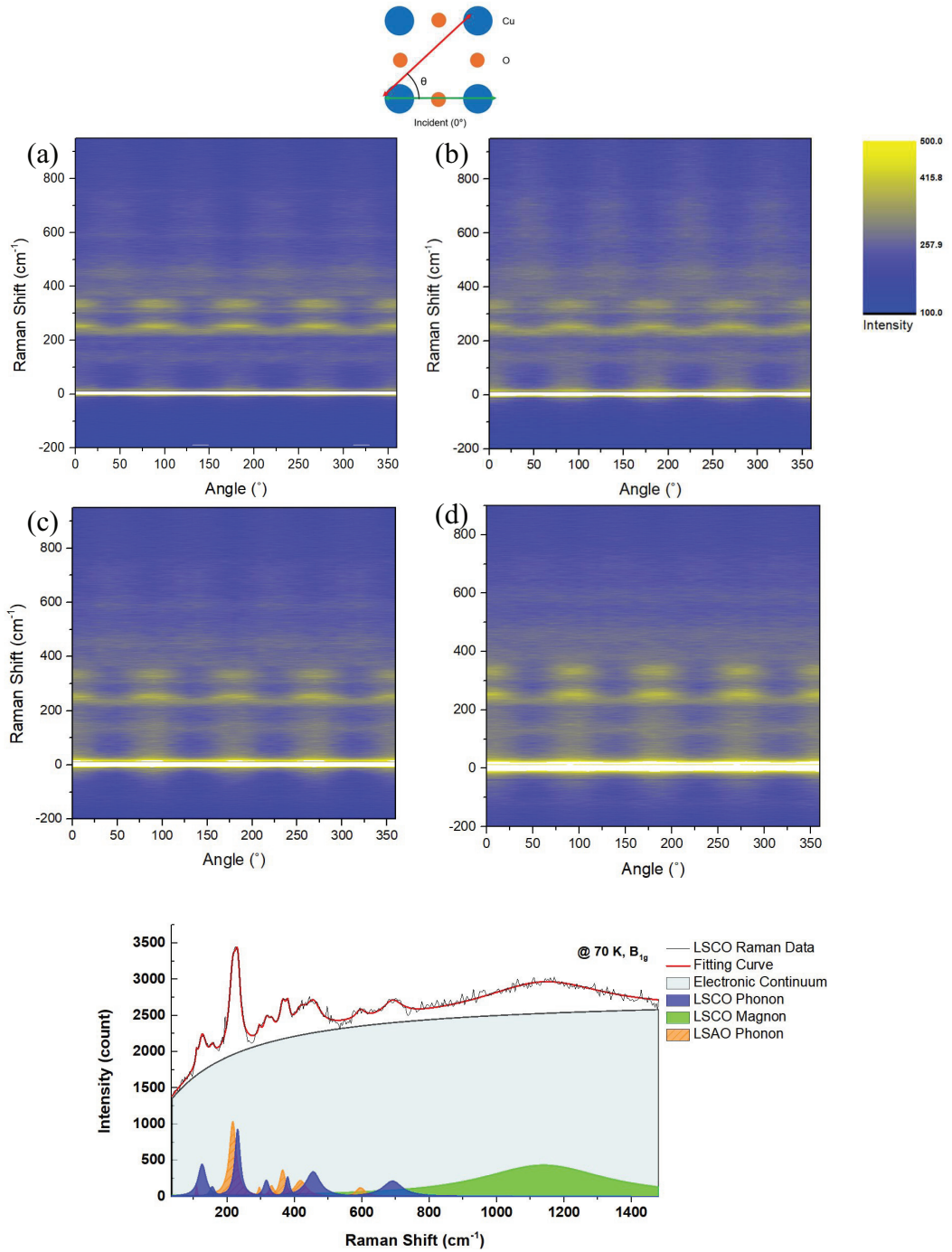


[그림 1] (a) 박막 $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ 샘플의 개략도. LSAO 기판 위에 펄스레이저를 통하여 LSCO를 증착하였다. LSCO 두께는 132 nm (100 unit cell)이다. (b) 실험에서 사용한 라만 분광 개략도. P는 편광기(Polarizer), HWP는 반파장판(Half Wave Plate)을 의미한다. 본 실험에서는 입사광과 산란광의 편광_방향이 서로 수직이다.

2-2-1. $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$ 박막의 라만 스펙트럼

그림 2 의 (a) ~ (d)을 보면 온전히 사중 대칭의 형태를 띠며, 온도에 따라서도 이중 대칭으로 바뀌지 않고 라만 데이터 상으로는 모든 영역에서 사중 대칭을 유지하는 것을 볼 수 있다. 이는 온도가 바뀌더라도 정방정계 구조이거나, 밑면의 양 변의 길이가 관측되지 않을 만큼 작은 차이를 지니는 사방정계 구조임을 추측할 수 있다. 또한 이를 통해 각각 B_{1g} 그리고 B_{2g} 데이터를 추출할 수 있다. Cu-O 결합에 45 도로 기울어진 빛으로 입사하고 산란된 빛 중 입사한 빛과 수직한 빛을 B_{1g} 데이터, Cu-O 결합에 평행한 입사하고 산란된 빛 중 입사한 빛과 수직한 빛을 B_{2g} 데이터로 수집을 하였다. 즉 그림 2 에서 각도상 50° 영역이 B_{1g} , 95° 영역이 B_{2g} 이다.

그림 2-(e)는 박막시료와 기관의 결과를 토대로 70 K 에서 LSCO B_{1g} 의 가공되지 않은 라만 스펙트럼에 대한 피팅을 진행한 결과이다. 포논과 2-마그논은 로렌츠 함수를, 전자 연속 스펙트럼은 기존 연구에서 개형을 확인한 후 다항 함수를 이용했고[10, 19, 20], 각 포논 피크의 위치는 시료의 데이터와 기관의 데이터를 비교하여 위치를 찾아냈다. 보여주는 것과 마찬가지로 $0\text{-}600\text{ cm}^{-1}$ 구간의 저 에너지 영역에서는 다수의 포논 피크가 존재하며, 이러한 포논은 기관의 포논 피크와 시료의 포논 피크가 겹쳐 있음을 확인할 수 있다. 이에 따라 박막의 시료의 데이터에서 기관의 데이터를 제거해야 온전히 LSCO 의 B_{1g} 에 대한 라만 데이터를 얻을 수 있음을 시사한다.

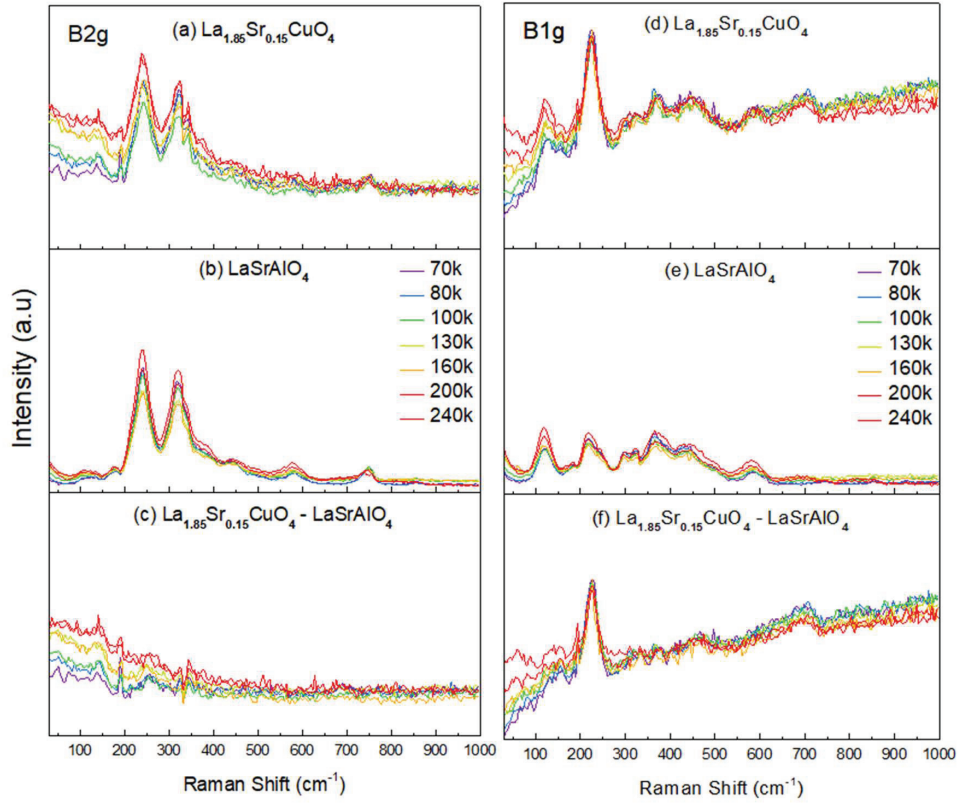


[그림 2] (a), (b), (c), (d) 각각 70 K, 100 K, 200 K, 300 K에서 입사광의 편광 각도에 따른 라만 스펙트럼 (e) LSCO 박막의 70K에서 B_{1g} 라만 스펙트럼 피팅

2-2-2. LSCO 박막의 라만 스펙트럼: 기판 데이터 제거 및 포논 분석

그림 3 의 (a)와 (b)를 보면 박막 시료와 LSAO 의 1000 cm^{-1} 영역까지의 가공되지 않은 데이터를 보여준다. 먼저 B_{2g} 데이터를 보면 박막 시료의 주요 피크는 전부 LSAO 즉 기판의 영향으로 나타난 것으로 보인다. 이는 기존에 라만으로 연구되었던 벌크 시료에서도 B_{2g} 포논 피크는 없는 것으로 알려져 있음과 일치한다[21]. 그림 3 의 (c)는 박막 시료의 데이터에서 기판의 라만 데이터를 뺀 데이터다. 기판은 같은 레이저 파워로 온도에 따라 조건을 같이 해서 측정하였으며, 이 때 라만 강도가 크기 때문에 기판의 라만 강도 데이터에 일정하게 같은 상수를 곱한 후 박막 데이터에서 뺐다. 일정 상수는 벌크 시료에서도, 본 실험에도 LSCO 박막의 포논 피크가 관측되지 않기에 뺀 데이터가 포논 피크가 보이지 않는 값으로 정했다[21].

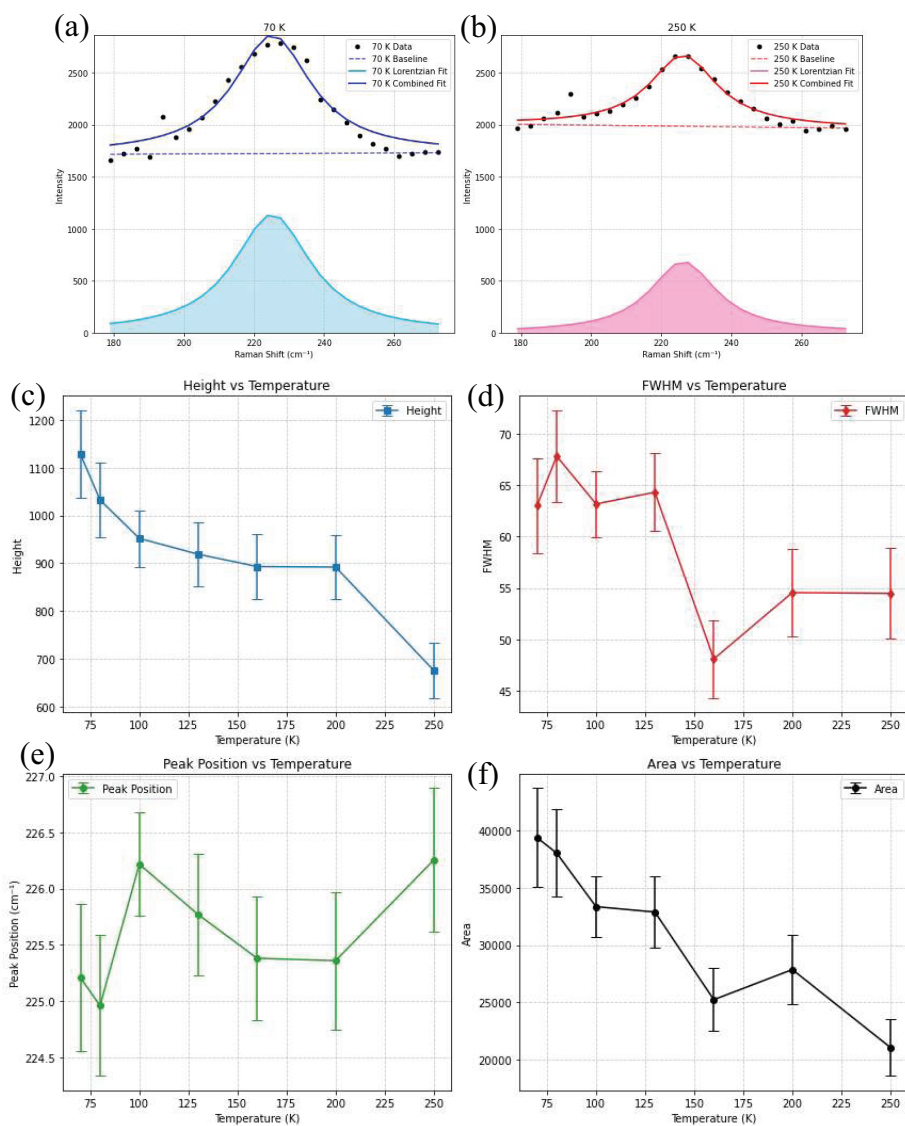
그림 3 의 (d) ~ (f)는 같은 방법으로 진행한 박막 LSCO 의 B_{1g} 데이터 값이다. 225 cm^{-1} 근방의 가장 큰 포논 피크가 하나 보이고, 그 이외에 작은 피크들이 450 cm^{-1} 과 700 cm^{-1} 근방에 존재하는 것을 볼 수 있다. 이러한 결과는 기존의 벌크 시료의 라만 데이터와 비교하였을 때 225 cm^{-1} 근방의 포논 피크가 기존의 벌크 시료의 라만 데이터와 차이가 있다. 벌크 시료에서는 그 근방의 포논이 두개의 피크로 갈라지지만, 본 실험에서는 하나의 피크로 보이는 것을 확인할 수 있다[21]. 이는 벌크 시료와 박막 시료 간에 라만 스펙트럼의 차이가 있다는 것이고, 이러한 차이에 대한 분석할 필요성이 있다.



[그림 3] (a) 박막 시료의 B_{2g} 라만 데이터 (b) 기판 LSAO B_{2g} 라만 데이터 (c) 박막 시료 데이터에서 기판 데이터를 뺀 데이터 (d) 박막 시료 B_{1g} 라만 데이터 (e) 기판 LSAO B_{1g} 라만 데이터 (f) 박막 시료 데이터에서 기판 데이터를 뺀 데이터

2-3. LSCO 박막의 포논 피크 분석

앞선 결과에서 LSCO 박막 시료의 라만 스펙트럼의 포논 피크 형태가 다르기에 이에 대하여 분석하였다. 앞서 나온 포논 피크는 로렌츠 함수로 피팅을 진행하였고, 피팅 하는 양 끝 점들의 평균을 낸 후 두 점을 잇는 선형 함수를 베이스 라인으로 정했다. 그림 4 (a), (b)을 보면 225 cm^{-1} 근방의 피크는 로렌츠 그래프를 가진다고 볼 수 있고, 이는 포논 잘 알려진 포논 피크의 형태이다[19]. 그림 4 의 (c) ~ (f)는 피팅한 포논 그래프를 온도에 따라 어떤 값을 지니는지 보여주는 그래프이다. 우선 그림 4(e)를 보면 피크의 중심은 오차범위 이내로 225.5 cm^{-1} 안팎의 값을 가진다. 온도에 따라 변화하긴 하지만 오차 범위 이내로 변화하는 것을 볼 수 있다. 또한 그림 4(c)를 보면 최고점과 포논의 넓이가 온도가 올라감에 따라 더욱 낮아지는 것을 볼 수 있다. 일반적으로 가공되지 않은 데이터의 경우 일반적으로 보즈-아인슈타인 응축의 영향으로 포논의 넓이와 최고점의 넓이가 온도가 증가함에 따라 증가한다[22]. 그러나 본 실험의 경우는 오히려 온도와 높이와 넓이가 반비례한 것을 알 수 있다. 이는 일반적으로 일어나는 현상이 아니며 이것이 LSCO 의 본래 성질인지 박막의 특성인지 후속 연구가 필요하다.

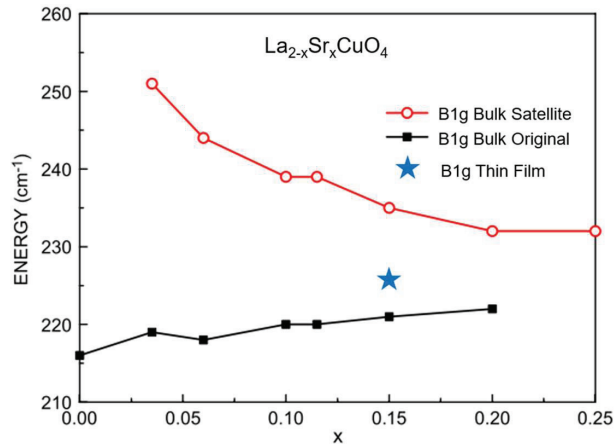


[그림 4] (a) ~ (b) 각각 70 K 과 250 K 에서서 180 ~ 270 cm^{-1} 에서 로렌츠 함수 피팅 결과 (c) 온도에 따른 로렌츠 함수의 최고점 높이 (d) 온도에 따른 로렌츠 함수의 반치폭 크기 (e) 온도에 따른 피크 중앙값 (f) 온도에 따른 로렌츠 함수의 넓이

2-4. 토론

본 연구에서 벌크 시료의 데이터와 가장 크게 다른 점은 B_{1g} 225.5 cm^{-1} 근방의 포논이 둘로 갈라지지 않고 하나로 보인다는 것이다. 왜 벌크 시료에서는 갈라지던 포논이 그렇지 않은 것일까? 벌크 시료를 측정한 논문들에 따르면 225 cm^{-1} 근방의 포논은 사방정계 구조로 인한 기원 피크와 기원 피크에서 파생된 위성 피크 둘로 나뉜다[21]. 그림 5는 Sr 도핑 농도에 따른 기원 피크와 위성 피크의 라만 이동 에너지 값을 나타내는 그래프다. 그림 5와 같이 기원 피크는 벌크 시료에서 도핑 농도가 전해질에 따라 에너지가 증가하다가 사라지고, 위성 피크는 도핑 농도가 전해질에 따라 에너지가 감소한다. 그러나 도핑이 없는 경우에는 위성 피크는 등장하지 않는다. 본 실험에서 측정한 도핑 농도 0.15의 박막 시료와 같은 경우는 기원 피크의 에너지와 위성 피크 에너지 그 사이에 존재한다. 벌크 시료를 측정한 논문들에 따르면 위성 피크는 국부적인 반전 대칭이 깨짐으로 인한 것으로 보고 있는데[21], 본 연구에서 등장한 포논 피크가 위성 피크가 아닌 기원 피크라면 이는 박막 제작 방식으로 만든 시료는 반전 대칭이 깨지는 현상이 없거나 더욱 적게 일어난다고 해석할 수 있다.

PLD로 제작한 박막 시료는 기존 벌크로 제작되면 플러팅 존 방식보다 더욱 높은 결정성을 지닌다. 이러한 높은 결정성은 더욱 낮은 불순도를 가지게 되고 원하지 않는 구간에 반전 대칭성이 깨지는 현상을 생각해볼 수 있다. 또한 벌크 시료에서 알려지기로는 저온에서 LSCO는 정방정계에서 사방정계로 결정성이 바뀐다고 알려져 있기도 했는데, 그림 2을 보면 온도가 저온과 고온에서 모두 사중대칭 보이는 것 또한 저온에서도 정방정계를 유지하거나 사방정계로 가는 정도가 라만으로 보이지 않을 정도로 작아진다고 해석할 수 있다. 이는 라만 분광학을 통하여서도 간접적으로 시료의 결정성을 확인할 수 있음을 시사한다. 또한 기존 벌크 시료와 박막 시료 간의 전기적 특성이 다른 것으로 알려져 있는데 포논 또한 차이가 있음을 보인다.



[그림 5] 도핑 농도에 따른 벌크 시료와 박막시료의 포논 에너지. 검은 선과 빨간 선은 각각 [21]논문에서 가져온 벌크 시료의 기원 포논과 위성 포논이다. 그리고 파란별은 본 실험의 박막 LSCO의 포논이다.

3. 결론

본 연구에서는 박막 LSCO에 대한 라만 스펙트럼을 분석하고 벌크 LSCO와의 비교를 통해 포논을 분석하였다. 박막 LSCO에 대한 각도 의존성 라만 스펙트럼을 분석한 결과 각도에 따라 사중대칭이 나타나는 것으로 보이며, 이는 박막 시료가 정방정계 구조에 속해 있거나 거의 정방정계에 가까운 사방정계 구조라고 추측할 수 있다. 또한 포논을 분석하며 시료의 라만 데이터에서 기관과 LSCO 박막의 데이터를 분리했다. 그 결과 기존 벌크 시료와 다르게 225 cm^{-1} 근방의 영역에서 포논 피크가 두개로 갈라지지 않고 하나의 피크로 존재하였다. 이는 박막과 벌크 시료 간에 라만 스펙트럼의 차이가 있다는 것을 의미하고, 박막에 대한 라만 연구의 필요성을 말한다. 또한 갈라지는 포논 피크가 등장하지 않은 것은 반전 대칭성이 깨지지 않거나 그 정도가 매우 약하다고 추측할 수 있는데, 펄스 레이저 증착 방식 특성상 불순도가 적기에 그렇다고 해석할 수 있다. 이러한 결과를 종합해보면 라만 분광학을 통하여 박막 시료를 연구할 수 있으며, 또한 시료의 결정 상태를 파악할 수 있음을 말한다.

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8 종의 전이 금속 디칼코게나이드(TMDs) 물질에 대한 라만 스펙트럼 분석

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전이 금속 디칼코게나이드(Transition Metal Dichalcogenides, TMDs)는 화학식이 MX_2 형태로 표현되는 화합물로, 여기서 M은 몰리브덴, 텅스텐, 하프늄, 주석과 같은 전이금속 원소이고 X는 황, 셀레늄과 같은 칼코젠 원소이다. 이들 물질은 층 수에 따라 다양한 밴드 구조를 갖는 특성 때문에 활발히 이루어지는 소재이다. 이황화몰리브덴(MoS_2)은 TMDs 중에서 광학적 연구가 가장 활발히 이루어진 물질이지만, 다른 TMDs에 대한 연구는 상대적으로 부족한 상황이다. 본 연구에서는 총 8종의 TMDs에 대한 라만 분석을 통해 다양한 TMDs 물질의 광학적 특성을 체계적으로 조사하였다. 실험 결과, 각 물질의 E_g , A_{1g} 모드의 피크 위치 변화가 층 수에 민감하게 반응함을 확인하였다. 이를 통해 TMDs의 층 수 판별 및 구조적 변화를 비파괴적으로 분석할 수 있음을 확인하였다.

키워드: 이차원 물질, 전이금속 디칼코게나이드, 라만모드

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I. 서론

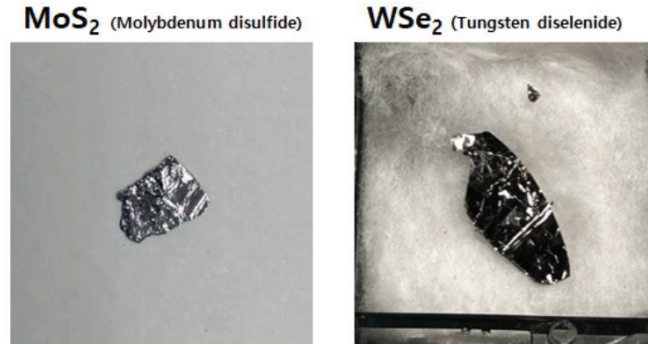
2004 년 안드레 가임과 콘스탄틴 노보셀로프는 테이프를 이용해 단일 원자 두께의 그래핀을 성공적으로 박리하여, 그래핀이 매우 높은 전기 전도성과 기계적 강도를 지닌 물질로 주목받게 되었다.[1] 그러나 그래핀의 전자 구조는 디랙 콘(Dirac cone) 형태를 가져 밴드 갭이 존재하지 않아 스위칭 특성이 요구되는 전자 소자 응용에 한계가 있었다.

이러한 한계를 극복하기 위해 연구자들은 밴드 갭을 가지면서도 2 차원 구조의 전이 금속 디칼코게나이드(Transition Metal Dichalcogenides, TMDs)에 주목하였다. TMDs 는 MX_2 (M: 전이금속, X: 칼코젠 원소) 형태를 가지며, 독특한 전기적 및 광학적 특성으로 차세대 소자 개발에 유망한 물질로 평가받고 있다.

TMDs 는 물질 내 결함이 물리적 특성에 큰 영향을 미치기 때문에, 결함의 유무와 구조-물성 관계를 파악하는 것이 중요하다. 라만 분광학은 비파괴적 방법으로 TMDs 의 물성을 연구하는데 유용한 도구로 널리 활용되고 있다. 현재 이황화몰리브데넘(MoS_2)은 TMDs 중 가장 활발히 연구된 물질로 꼽히지만, 상대적으로 다른 TMDs 물질들에 대한 광학적 연구는 제한적이며, 이들 물질의 특성을 심층적으로 이해하기 위한 연구가 요구된다.

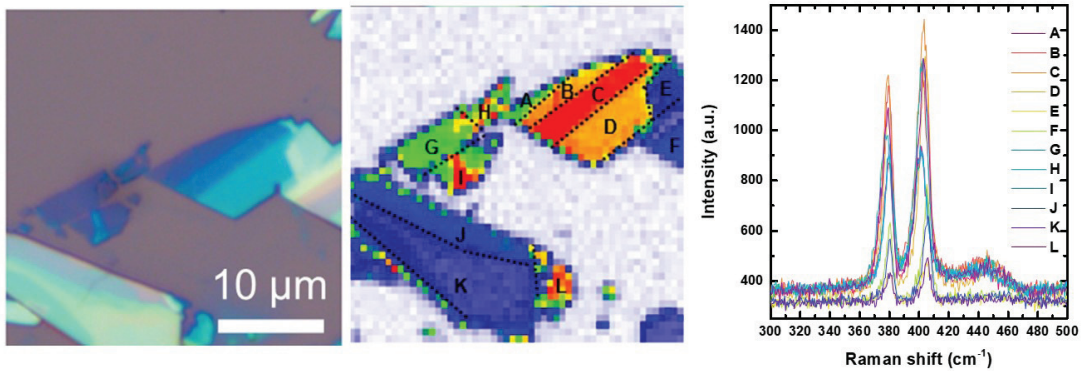
본 연구에서는 8 종의 TMDs 물질에 대해 라만 분광을 측정하여, 각 물질의 특유한 광학적 및 진동 특성을 분석하였다. 이를 통해 TMDs 물질의 구조-물성 관계에 대한 이해를 심화시키고, 차세대 전자 및 광학 소자 개발에 중요한 기반을 제공할 것으로 기대된다.

II. 본문



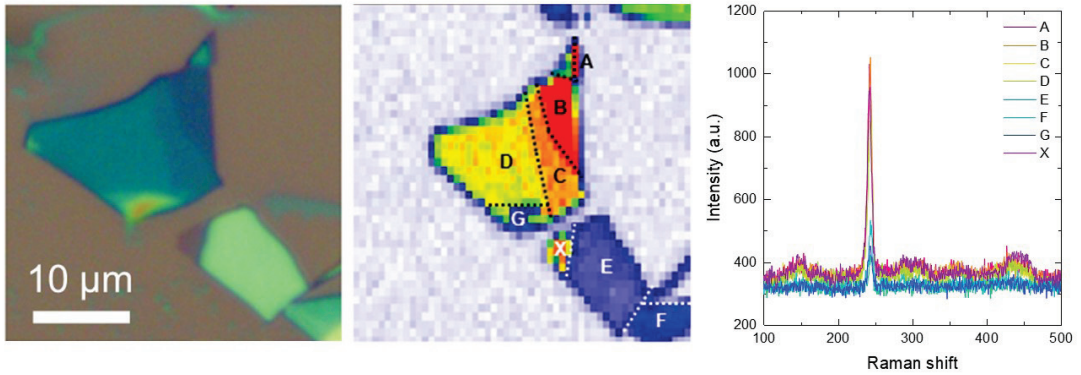
[그림 1] 이황화몰리브덴과 아셀렌늄화텅스텐 결정 사진

그림 1은 본 실험에 사용된 TMDs 결정이다. 각각의 결정들은 접착 테이프를 이용하여 기계적으로 박리되었다. 이 박리 방법은 원자 층 두께 수준으로 물질의 층간 분리가 가능하다. 박리 후 물질이 묻어 있는 테이프는 실리콘 (Si)/이산화 규소 (SiO₂)면이 있는 기판 중 약 300 nm 두께의 이산화규소면에 전사되었다. 또한 실험에 사용된 모든 기판은 초음파 세척기를 이용하여 아세톤에서 약 30 분 정도 세척되었으며, 이후 아이소프로필알코올을 이용하여 세척 후 에어건을 이용하여 건조하였다. 기판에 전사된 물질들 중 실험에 사용할 시료를 선별하기 위해서 광학현미경을 이용하였다. 라만 분광학 측정의 경우 532 nm 의 발진 레이저와 100배 대물렌즈를 이용하였다. 측정은 약 0.4 mW 이하의 세기에서 진행되었으며, 노출 시간은 0.5 초로 설정하였다.



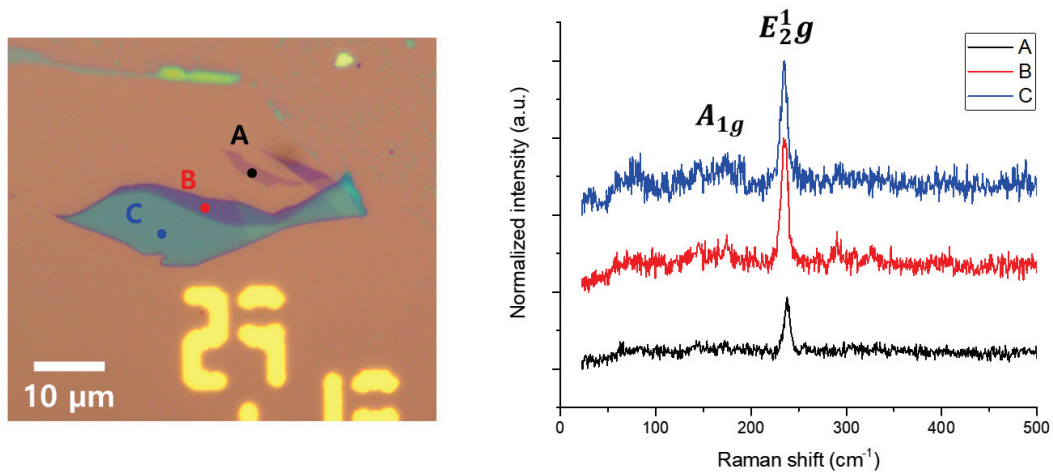
[그림 2] 이황화몰리브덴의 사진과 라만 라만 스펙트럼 측정 결과

그림 2는 이황화 몰리브덴 (molybdenum disulfide, MoS_2)의 사진 및 라만 스펙트럼 측정 결과이다. MoS_2 은 반도체적 성질을 가지며 수 층 이상에서는 약 1.2 eV의 간접 밴드갭을, 단 층 수준일 때 1.9 eV의 직접 밴드갭을 가진다.[2,3] 층 수에 따라 밴드갭의 변화 뿐만 아니라 라만 분광 스펙트럼의 모양도 달라진다. 532 nm의 레이저를 사용하였을 때 MoS_2 에서 일반적으로 두 개의 라만 신호를 확인할 수 있다. E_{2g} 모드는 물질에서 평면 내 (in-plane) 진동하는 모드로, 몰리브덴 원자와 황 원자가 평면 내에서 서로 반대 방향으로 진동하여 발생된다. 이 모드는 약 383에서 385 cm^{-1} 근처에서 관찰되며 층 수가 증가할수록 감소하게 된다. 또한 A_{1g} 모드는 물질에서 평면 밖 (out-of-plane)에서 진동하는 모드로 황 원자가 몰리브덴 원자를 기준으로 평면과 수직한 상하 방향으로 진동하여 나타나는 모드이다. 이 모드는 약 403에서 405 cm^{-1} 근처에서 관찰되며, 반대로 층 수가 증가함에 따라 같이 증가하게 된다. 물질 내에서 층간 상호작용에 의해서 나타나는 E_{2g} 와 A_{1g} 라만 모드 신호는 단층일 때 거리가 가장 가까우며, 두께가 증가할수록 간격이 벌어지게 된다. 이 차이를 이용하여 물질의 정확한 층 수를 구별할 수 있게 된다.



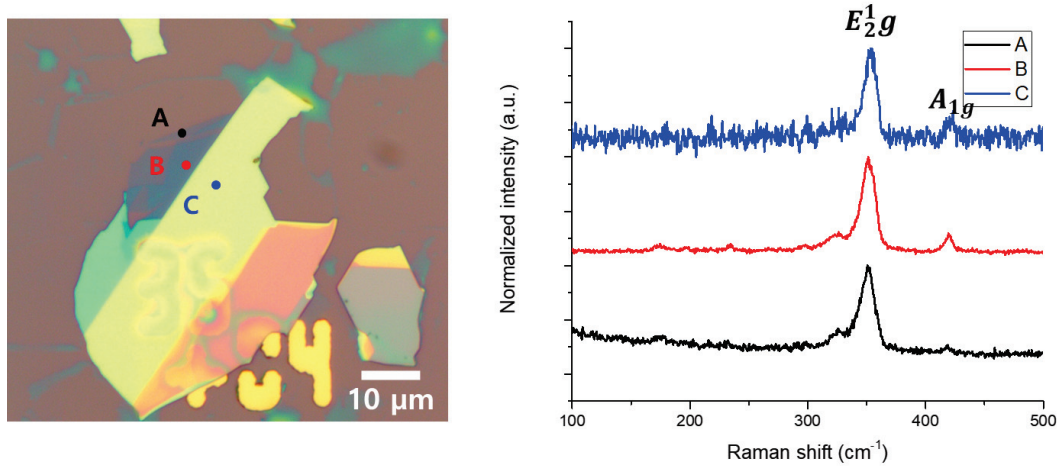
[그림 3] 이셀레늄화몰리브덴의 사진과 라만 스펙트럼 측정 결과

그림 3은 이셀레늄화 몰리브덴 (molybdenum diselenide, MoSe_2)의 사진 및 라만 스펙트럼 결과이다. MoSe_2 도 MoS_2 와 마찬가지로 몰리브덴과 셀레늄 사이는 공유결합을, 층 간에는 판데르발스 결합을 하고 있어서 접착 테이프로도 쉽게 박리가 가능하다. MoSe_2 의 라만 스펙트럼의 경우 532 nm 레이저로 여기시켰을 때 100에서 400 cm^{-1} 근처에서 다양한 라만 모드 신호가 나타나며 특히 약 244 cm^{-1} 에서 A_{1g} 모드에 해당하는 신호를 확인할 수 있다.[4]



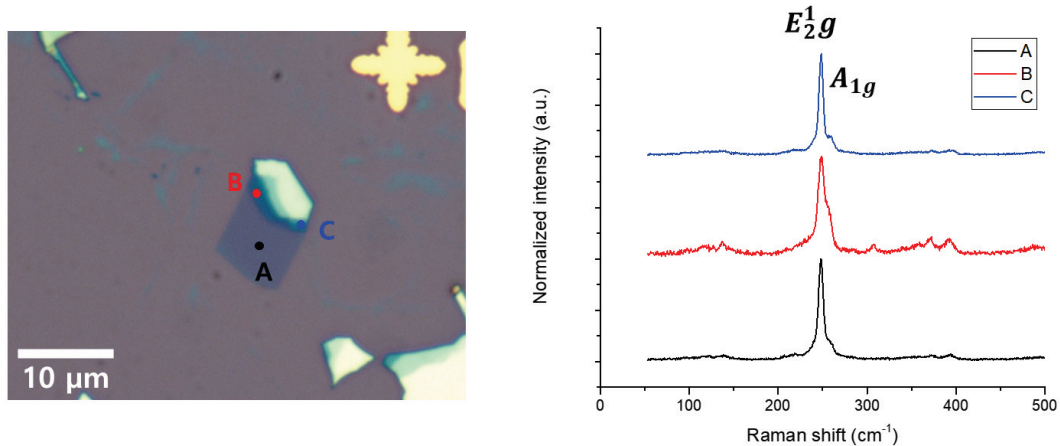
[그림 4] 이텔루륨화몰리브덴의 사진과 라만 스펙트럼 측정 결과

그림 4는 이텔루륨화 몰리브덴 (molybdenum ditelluride, MoTe_2)의 사진과 각 위치에서의 라만 스펙트럼이다. MoTe_2 의 라만 스펙트럼에서는 일반적으로 A_{1g} 모드, E'_{2g} 모드가 관찰된다. A_{1g} 모드는 약 170 cm^{-1} 에서 나타나며 텔루륨 원자들이 결정 평면과 수직 방향으로 진동하는 모드이다. E'_{2g} 모드는 약 234 cm^{-1} 에서 나타나며, 몰리브덴과 텔루륨 원자들이 결정 평면과 평행한 방향으로 진동하는 모드이다.[5] 특히 E'_{2g} 모드는 MoTe_2 의 층 수가 증가함에 따라 피크 위치가 이동하며 라만 시프트가 감소하는 방향으로 이동하는 적색편이가 관측된다. 그림에서 각각 다른 두께의 MoTe_2 를 측정 한 A, B, C에서도 E'_{2g} 모드의 적색편이가 확인된다. 따라서 라만 분광법을 이용하면 MoTe_2 의 층 수를 비파괴적으로 분석할 수 있다.



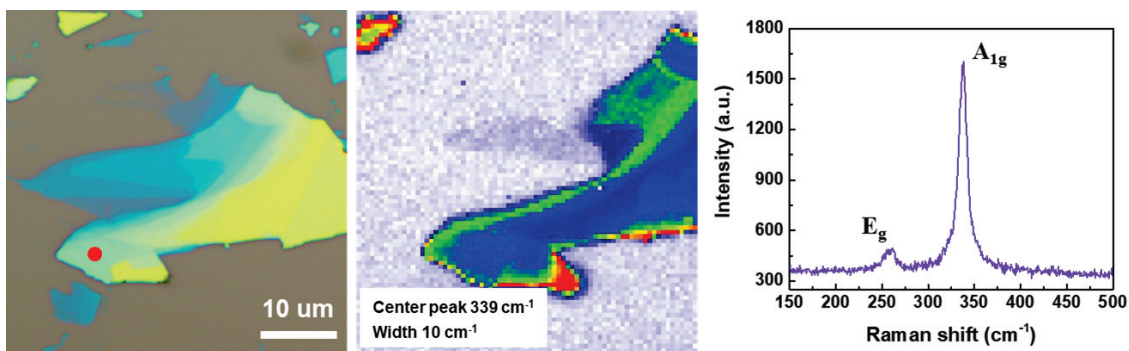
[그림 5] 이황화텅스텐의 사진과 라만 스펙트럼 측정 결과

그림 5는 이황화 텅스텐 (tungsten disulfide, WS_2)의 사진 및 라만 스펙트럼 결과이다. WS_2 의 라만 스펙트럼에서는 주로 E'_{2g} 모드와 A_{1g} 모드가 관찰된다. E'_{2g} 모드는 약 357 cm^{-1} 에서 나타나며 A_{1g} 모드는 약 418 cm^{-1} 에서 나타난다.[6] 층 수에 따라 E'_{2g} 모드와 A_{1g} 모드의 피크 위치가 이동되며 그림 5의 그래프에서도 A_{1g} 모드 피크의 위치가 청색편이 된다. 따라서 MoTe_2 에서와 동일하게 비파괴적으로 층 수를 분석할 수 있다.



[그림 6] 이셀레늄화텅스텐의 사진과 라만 스펙트럼 측정 결과

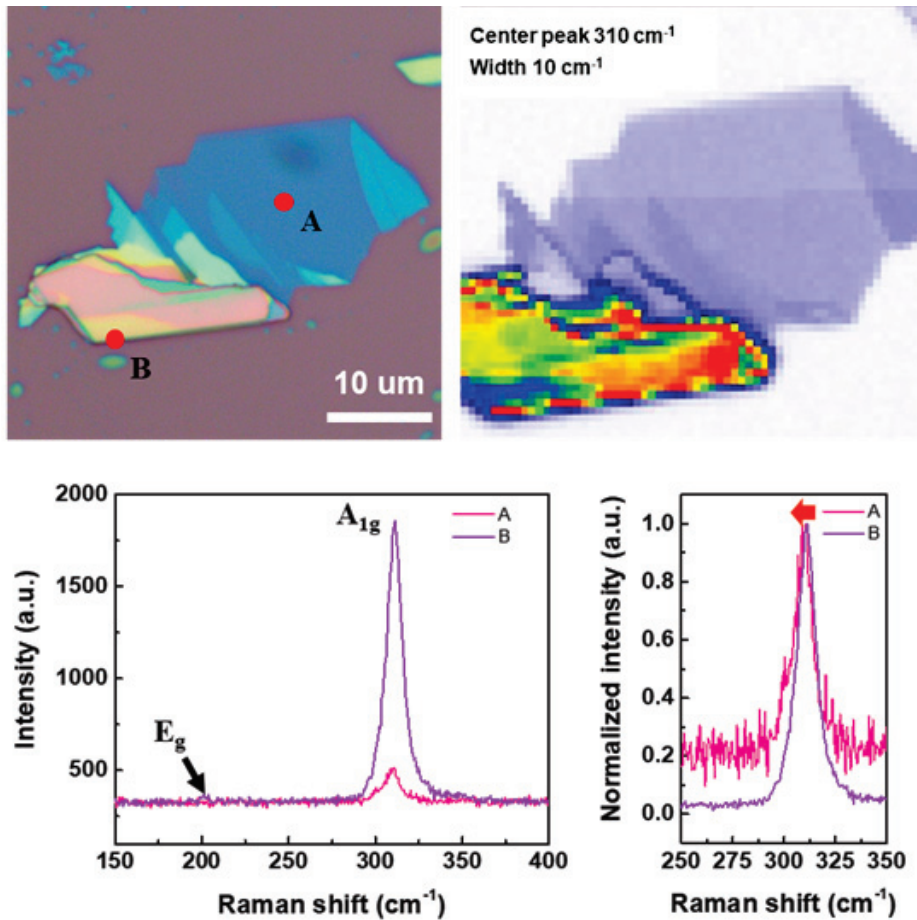
그림 6는 이셀레늄화 텅스텐 (tungsten diselenide, WSe_2)의 사진 및 라만 스펙트럼 결과이다. WSe_2 의 라만 스펙트럼에서는 주로 E_{2g}^1 모드와 A_{1g} 모드가 관찰된다. E_{2g}^1 모드는 약 248 cm^{-1} 에서 나타나며 A_{1g} 모드는 약 251 cm^{-1} 에서 나타난다.[7,8] E_{2g}^1 모드와 A_{1g} 모드 역시 층 수에 다른 피크 위치 이동을 확인할 수 있다.



[그림 7] 이황화 하프늄의 사진과 라만 스펙트럼 측정 결과 및 맵핑 이미지

그림 7는 이황화 하프늄 (Hafnium Disulfide, HfS_2)의 사진 및 라만 스펙트럼 측정 결과이다. HfS_2 의 라만 스펙트럼에서 A_{1g} 모드(338.5 cm^{-1})와 E_g 모드(260.2 cm^{-1})가 관측되었다.[9] A_{1g} 모드는 황 원자의 평면 밖(out-of-plane) 진동에 주로 영향을 받으며, E_g 모드는 평면 내(in-plane)

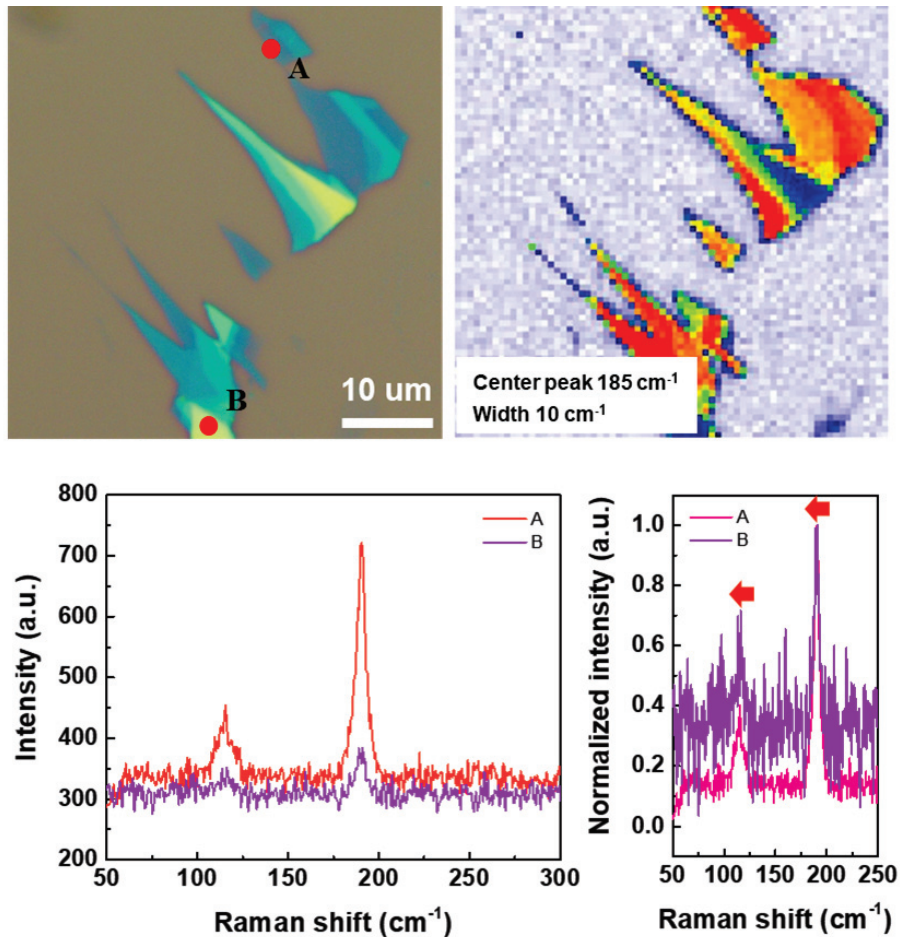
진동으로 두께 변화 및 압력에 민감하게 반응한다. 특히, A_{1g} 모드의 라만 신호로 생성된 맵핑 이미지를 분석한 결과, 샘플 두께가 얇은 영역에서는 라만 신호 강도가 감소하여 해당 부분이 희미하게 나타났으며 맵핑 이미지는 실제 시료의 이미지와 잘 일치한다.



[그림 8] 이황화 주석의 사진과 라만 스펙트럼 측정 결과 및 맵핑 이미지

그림 8은 이황화 주석 (Tin Disulfide, SnS_2)의 사진 및 라만 스펙트럼 측정 결과이다. SnS_2 의 라만 모드는 E_g 모드(203.9 cm^{-1})와 A_{1g} 모드(311 cm^{-1})가 관측되었다. E_g 모드는 평면 내(in-plane) 비등방성에 영향을 받고, A_{1g} 모드는 황 원자의 평면 밖(out-of-plane) 진동에 의존한다. A_{1g} 모드는 두께가 감소함에 따라 피크의 진동수가 적색편이 되고 강도가 약해지는 경향이

관찰되었으며, 이는 이전에 보고된 이론적 계산 결과와 일치한다.[10] 또한, 중심 파장을 310 cm^{-1} 로 설정해 라만 맵핑한 이미지는 광학 현미경으로 측정한 이미지와 일치한다.



[그림 9] 이셀렌화 주석의 사진과 라만 스펙트럼 측정 결과 및 맵핑 이미지

그림 9 는 이셀렌화 주석 (Tin Diselenide, SnSe_2)의 사진 및 라만 스펙트럼 측정 결과이다. SnSe_2 의 라만 피크는 E_g 모드(119.4 cm^{-1} , bulk 기준)와 A_{1g} 모드(191.0 cm^{-1} , bulk 기준)로 측정되었으며, 두께가 감소함에 따라 E_g 모드와 A_{1g} 모드 모두 라만 피크가 적색편이 되는

경향을 보였다. 이러한 결과는 이론적 계산과 잘 부합하는 것으로 확인되었다. [10] 또한, 중심 파장을 185 cm^{-1} 로 설정해 라만 맵핑한 이미지는 광학 현미경 이미지와 일치한다.

III. CONCLUSION

본 연구에서는 이차원 물질 중 반도체적 성질을 가지는 전이금속 디칼코게나이드 물질의 라만 분광 특성을 분석하였다. MoS_2 를 포함한 8종의 물질에 대해 라만 진동 모드의 위치 및 세기 변화를 관찰하였으며, 이를 통해 각 물질의 고유한 광학적 특성을 확인할 수 있었다. 특히, 물질의 층 수 변화에 따른 라만 신호의 변화를 관찰함으로써, 물질의 층 수를 정확하게 관별할 수 있는 비파괴적 분석법을 확립하였다. 향후 단일 물질의 결함 특성 및 이종 구조 분석으로 연구를 확장하여 전이금속 디칼코게나이드 계열의 물질의 응용 가능성을 더욱 확대할 수 있을 것으로 기대된다.

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Chronic exposure to low dose of ethanol induces hepatic malfunction in *Danio rerio* and *Oryzias sinensis*

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Abstract

Ethanol is widely recognized as a hepatotoxin, with the liver serving as the primary target for toxicity due to its central role in ethanol metabolism. In this study, significant alterations in hepatic indicators were examined in two fish species, *Danio rerio* and *Oryzias sinensis*, exposed to sublethal concentrations of waterborne ethanol (0, 0.1, 0.5, and 1%) for 50 days. While no significant effects on the hepatosomatic index were observed in either species, prominent red lipid droplets, unclear cellular boundaries, and cellular swelling were evident in liver tissues from both species. Additionally, significant increases in the enzymatic activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase at 0.5% and/or 1% ethanol exposure suggested impaired hepatic function following prolonged exposure. These findings indicate that consistent exposure to sublethal concentrations of ethanol is detrimental to liver function in fish.

1. Introduction

Alcoholic liver disease refers to liver dysfunction caused by chronic alcohol consumption, encompassing a spectrum of conditions including fatty liver, liver fibrosis, and liver cancer. Among these, alcoholic fatty liver disease—induced by alcohol intake—cannot be diagnosed visually and exhibits significant variability in symptoms and severity among individuals. Furthermore, the progression of fatty liver and steatohepatitis remains poorly understood in fish, and research into alcoholic liver disease in aquatic organisms is still ongoing. Globally, numerous chemicals, including ethanol (EtOH), are known to impact various aquatic organisms. However, assessing environmental effects on every species is impractical, so model organisms are utilized for impact evaluations. Scientific evidence derived from fish model species is often extrapolated to other organisms and is particularly valuable in human disease research as an alternative to direct human experimentation.

Ethanol exposure affects fish behavior, physiology, and stress responses in dose-dependent ways, providing insights into its broader harmful effects (Fujisawa et al., 2019). For example, higher ethanol concentrations impair motor coordination, leading to immobility and reduced swimming speeds, indicative of its depressive effects on the central nervous system (Araujo-Silva et al., 2018). Prolonged exposure during developmental stages in zebrafish has been linked to persistent anxiety-like behaviors, suggesting neurodevelopmental impacts comparable to those observed in mammals (Baiaamonte et al., 2016). Furthermore, ethanol exposure alters predator perception and stress responses, as seen in zebrafish, where it reduces cortisol-mediated stress responses and disrupts communication of predation risk (Oliveira et al., 2013; Schneider et al., 2017). These findings highlight the utility of fish models, such as zebrafish, in studying ethanol's neurobehavioral effects, providing insights into addiction and stress regulation mechanisms.

In this study, we induced alcoholic liver disease in *Danio rerio* and *Oryzias latipes* with chronic exposure to sub-lethal concentrations of EtOH to compare morphological differences in fatty liver development. Through this comparison, we aimed to evaluate the suitability of fish model species for studying the progression of liver diseases. Ultimately, this research seeks to highlight the potential of fish models not only for investigating liver diseases, including alcoholic liver disease in humans, but also for studying liver pathologies in fish.

caused by marine environmental changes and pollution.

2. Main

The *D. rerio* used in the experiment were purchased from Mariacua (Namyangju, Republic of Korea), with an average size of 3.45 cm (n = 6). The zebrafish were maintained in an incubator (MIR-554-PE; Panasonic Holdings Corporation, Osaka, Japan) at 28°C under a light cycle of 14 hours light / 10 hours dark for 50 days. For feeding, 3 ml of *Artemia* nauplii (SEP-Art; Ocean Nutrition, Essen, Belgium) were provided daily.

The *O. sinensis* used in the experiment were purchased from Greenfish (Seoul, Republic of Korea), with an average size of 2.86 cm (n = 6). The rearing conditions for the medaka were the same as those used for the zebrafish.

In the ethanol exposure experiment for the experimental fish, both *D. rerio* and *O. sinensis* had control groups that were not exposed to ethanol (n = 3), and experimental groups that were treated with a 1% ethanol concentration (n = 3). Both the experimental and control groups were reared for 50 days in 8 L non-circulating tanks. The 1% ethanol concentration for the experimental group was prepared by adding 80 mL of ethanol using conical tubes. To account for ethanol evaporation and prevent a decrease in ethanol concentration in the tanks, daily water changes were performed, and the tanks were covered with acrylic plates.

After 50 days, the *D. rerio* and *O. sinensis* were euthanized, and their livers were collected. The HSI was calculated as the ratio of total liver weight to total body weight, using the following formula: (liver weight/body weight) × 100. The collected livers were divided into two parts: one was processed for paraffin embedding and the other for frozen embedding. For paraffin embedding, liver tissue was fixed in 4% formaldehyde (Sigma-Aldrich, Darmstadt, Germany). For frozen embedding, liver tissue was processed into OCT blocks using cryomolds and FSC22 Frozen Section Comp Clear (Leica Biosystems, Nussloch, Germany) in a VIP Series ultra-low temperature upright freezer (Panasonic Holdings Corporation, Osaka, Japan) at -80°C.

Liver tissue fixed in 4% formaldehyde solution for one day was placed in cassettes and rinsed with running tap water for one day. After rinsing, the tissue was dehydrated using an

automatic tissue processor (Excelsior-AS; Thermo Fisher Scientific, Runcorn, UK) by treating it with ethanol in a gradient from low to high concentration. Then, xylene was used to clear the ethanol and xylene, followed by paraffin infiltration to prepare the tissue for paraffin block formation. After the tissue processing, paraffin blocks were made using a tissue embedding machine (Histostar; Thermo Fisher Scientific, Runcorn, UK). The paraffin blocks were sectioned using a microtome (HM355S; Thermo Fisher Scientific, Massachusetts, U.S.), and the sections were placed in a water bath at a controlled temperature to flatten the tissue before mounting it onto slides. To ensure adhesion to the slides, the sections were dried and then treated in an oven (58°C–60°C).

Hematoxylin and eosin (H&E) staining was carried out using an automatic stainer (HMS 70; Videotron Corporation, Osaka, Japan). To remove the paraffin from the tissue, xylene was used, followed by ethanol treatment in a gradient from high to low concentrations to remove water from the tissue. After staining with hematoxylin, eosin staining was performed in sequence. Finally, the tissue was treated with ethanol and xylene for preservation.

In the pre-processing step, the tissue-containing OCT block was fixed in a tissue cryostat (HM520; Hanwha, Seongnam-si, Republic of Korea), and sections were cut. The resulting sections were placed onto slides and dried. After drying, the sections were fixed in formalin and then rinsed. The water inside the tissue was removed using isopropanol, and Oil Red O staining and hematoxylin staining were performed. The stained slides were observed under a light microscope (ECLIPSE Ni-U; Nikon, Tokyo, Japan).

The enzymatic activities of ALT, AST, and ALP in liver tissues were measured using commercial kits (Fish ALT, AST, and ALP ELISA Kits, MyBioSource, Inc., San Diego, CA, USA), following the manufacturer's instructions (Nam et al., 2020). Briefly, ALT and AST activities were quantified colorimetrically with the color reagent 2,4-DNPH in the supernatant obtained from tissue homogenates. A 50 μ L aliquot of the supernatant was mixed with alanine- α -KG and aspartate- α -KG substrate buffers, respectively, and incubated at 37 °C for 60 minutes. The enzymatic activity was measured at 505 nm using a Varioskan Flash fluorometer (Thermo Fisher Scientific). For ALP, 50 μ L of the supernatant was added to a chromogen substrate, and the activity was assessed at 510 nm using the same fluorometer.

Firstly, no significant effect of EtOH treatment on HSI was observed in both fish at any concentration after 50 days exposure ($P > 0.05$) (Figure 1).

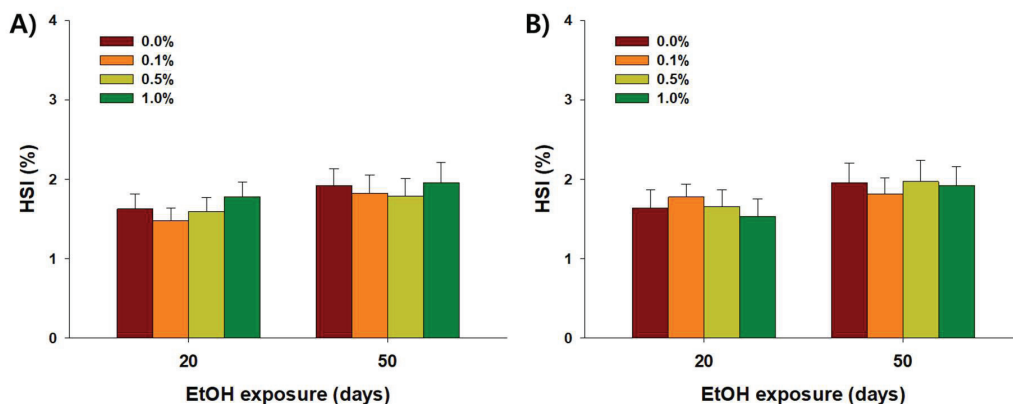


Figure 1. Results on effect of EtOH on HSI value of *Danio rerio* and *Oryzias sinensis*.

In the liver tissue images of the experimental group *D. rerio* (scale bar 50 μm , 40x magnification), red lipid droplets are observed throughout (Figure 2B). Additionally, there is uncertainty in the boundaries of liver cells and swelling of the cells (Figure 2D). In contrast, in the liver tissue images of the control group zebrafish, red lipid droplets are not observed (Figure 2A). Furthermore, the boundaries of liver cells are clearly defined, and there is an increased density of liver cells (Figure 2C).

In the liver tissue images of the experimental group *O. sinensis* (scale bar 50 μm , 40x magnification), red lipid droplets are observed throughout, and the size of the lipid droplets is large (Figure 3B). Additionally, the microvessels inside the liver tissue are dilated, and the boundaries of the liver cells are unclear (Figure 3D). In contrast, in the liver tissue images of the control group, the lipid droplets are smaller and observed at a lower frequency compared to the experimental group (Figure 3A). Furthermore, the degree of microvessel dilation is lower, and the density of liver cells appears increased (Figure 3C).

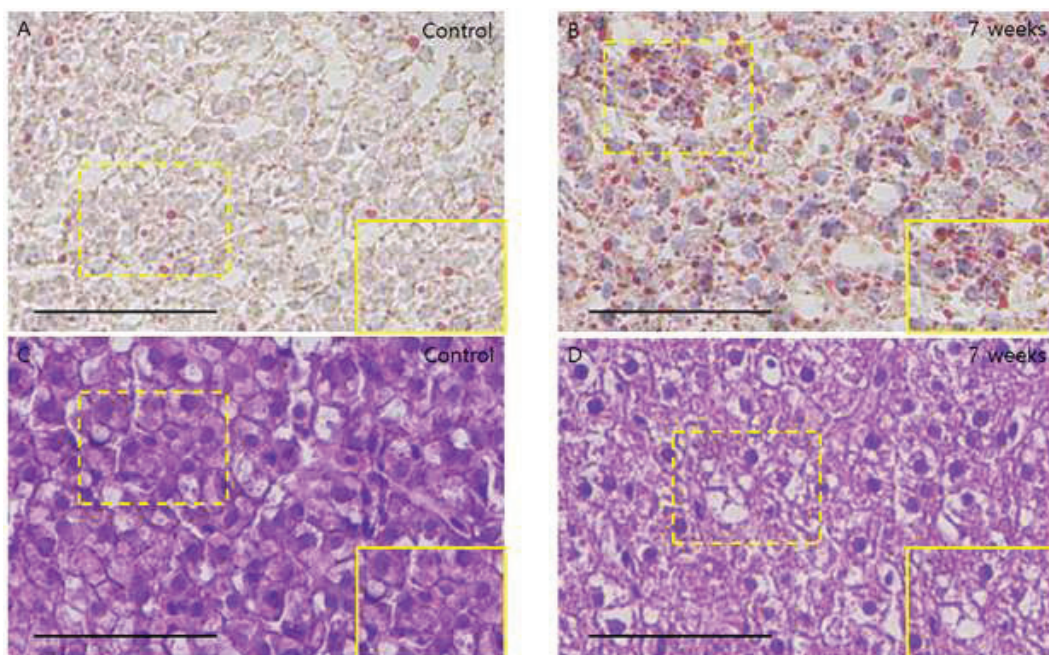


Figure 2. Results on effect of EtOH on the liver tissue of *Danio rerio*.

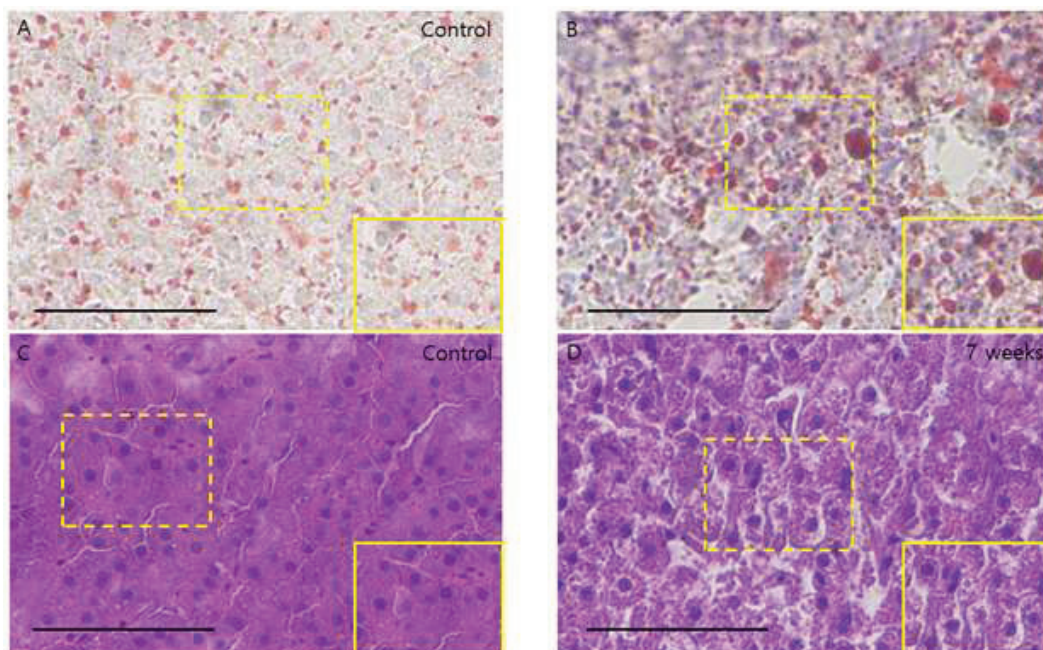


Figure 3. Results on effect of EtOH on the liver tissue of *Oryzias sinensis*.

Treatment with 0.5 and/or 1 % of EtOH significantly increased ALT, AST, and ALP activities of both *D. rerio* and *O. sinensis* on day 50 ($P < 0.05$) (Figure 4). Significant increases in the hepatic enzymatic activities support fluctuated hepatic function in both fish (Fujisawa et al., 2019).

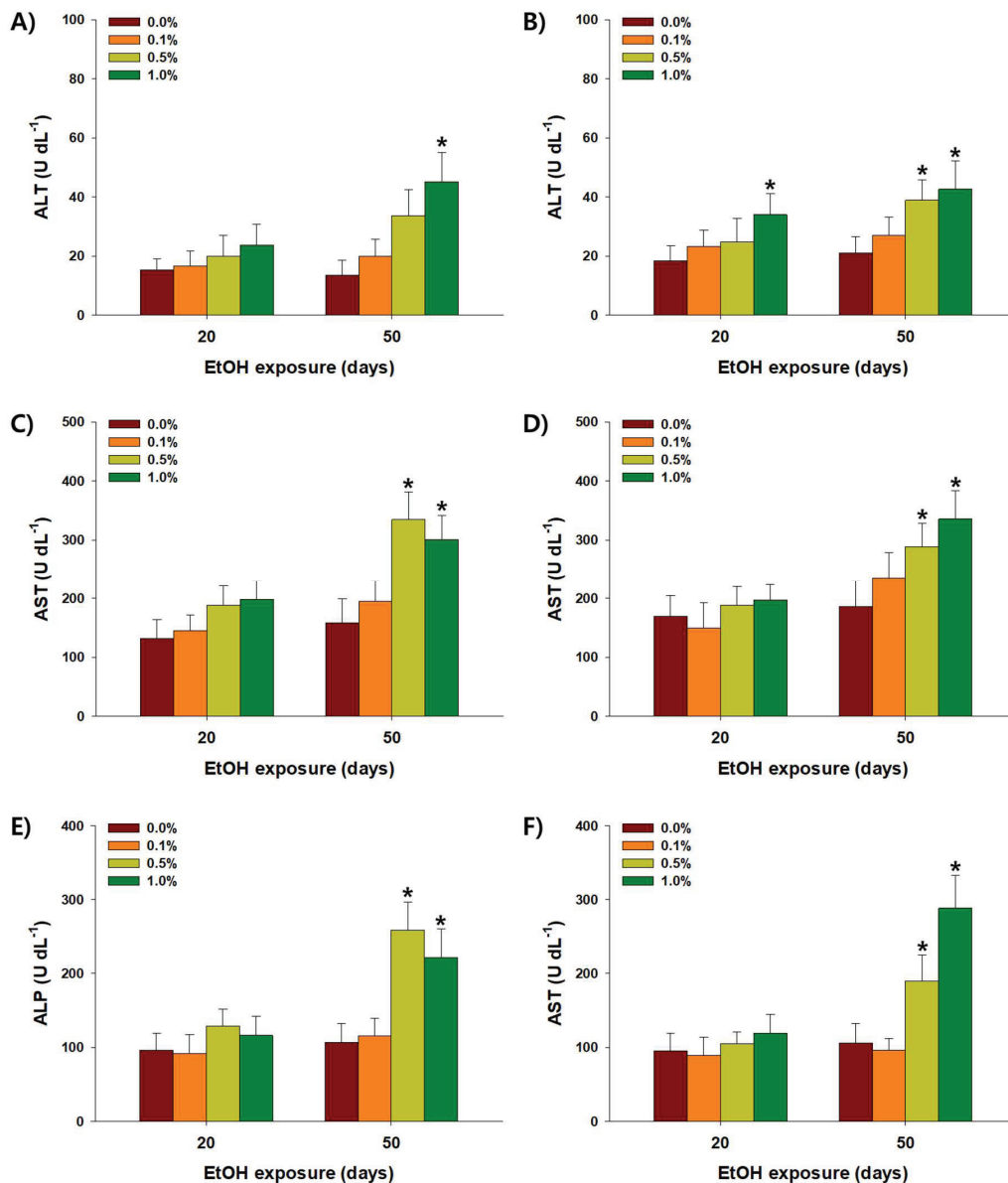


Figure 4. Results on effect of EtOH on the liver tissue of *Oryzias sinensis*.

The formation of fatty liver causes the normal liver cells to expand, and as a result of this expansion, the liver cells are destroyed (Orman et al., 2013; Lin et al., 2015). Repeated cycles of destruction and regeneration prevent the liver cells from performing their normal functions, leading to liver fibrosis (Schneider et al., 2017). This fibrosis ultimately progresses to cirrhosis or liver cancer. Both fatty liver and liver fibrosis can be treated if identified early, but cirrhosis and liver cancer are often untreatable, even if identified. For this reason, it is crucial to study the progression of these diseases using experimental species that can address both efficiency and ethical concerns, to understand the characteristics of lesions through the formation process of these diseases.

3. Conclusion

Zebrafish *D. rerio* are widely used as an experimental species due to their small size and ease of breeding. Additionally, because they share a high degree of genetic similarity with humans, they are commonly used to observe the development of pathological conditions, such as alcoholic liver disease. Similarly, *O. sinensis*, which is also widely used as an experimental species for the same reasons, has been shown to develop alcoholic liver disease. Additionally, the *O. sinensis* used in the experiment, shares similar characteristics with zebrafish, such as small size and ease of breeding. This study explored the potential use of the medaka as a domestic model species for observing pathological conditions. In the chronic ethanol exposure experiment (1% ethanol concentration, 50 days treatment) conducted on both zebrafish and Japanese medaka, both experimental species exhibited accumulation of fat within liver cells, vascular dilation, and expansion of liver cells in the ethanol-treated groups. As a result, the observation of alcoholic liver disease in both zebrafish and Japanese medaka, along with their small size, low cost, ease of breeding, and low maintenance requirements, suggests the potential of these species to be used as model organisms for studying the development of pathological conditions.

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SERS-based PCR for the monitoring of Klebsiella pneumoniae

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Surface enhanced Raman Scattering (SERS) has been widely spread for monitoring chemical and biological analytes due to its potential of multiplexing ability and high sensitivity. However, the last requirement of reproducibility remains one of the biggest challenges in the SERS-related analytical fields where target concentration is extremely low.

In this study, we described the integration of SERS with PCR for the detection of *Klebsiella pneumonia* which is a widely spread nosocomial pathogen. Basically, PCR has been used for the detection of pathogenic nucleic acid as a standard tool in the hospital to distinguish type of pathogens. However, their limitation was that it is difficult to analyze multiple target-genes at the same time from single reaction because it requires sets of excitation and emission wavelengths for the each of fluorophore that represent amplified DNA. It makes a challenge in terms of multiplex monitoring. In our research, we integrated PCR with SERS-based analytical approach because PCR can be used to amplify disease-related pathogen-specific sequences and SERS has a potential to determine various SERS probes at the same time. Here we have developed PCR-based SERS analytical approach for monitoring pathogen bacteria, *Klebsiella pneumonia*, because it is one of the most common pathogens that found from septic patient and it open cause severe medical condition if patient can be treated in an early infection status. That is why it requires rapid and sensitive detection tools for personalized medication in clinical setups. It is also well-known as food-borne bacteria which precise monitoring is required in food industries. More specifically, silver (Ag) and gold (Au) hybrid nanoparticles were synthesized and Raman probe-conjugated DNA-based 'structure switching sensors' were immobilized on it. Then, *Klebsiella pneumoniae*-specific sequence was amplified with asymmetric PCR approach, and it was detected using Ag-Au hybrid SERS sensors. Here, we demonstrated that single cell detection of *Klebsiella pneumoniae* using integrated PCR-based SERS. This platform technology will provide potential for multiplex monitoring of pathogens at a single cell sensitivity.

Keywords: SERS, Multiplex, PCR, *Klebsiella pneumoniae*

1. 서론

박테리아는 지구상에서 다양한 동·식물과의 공생을 통해 번식하고 질소를 순환시키는 등 다양한 순기능을 하고 있으나, 동시에 일부 박테리아는 병원균으로 작용하며 다양한 질병을 유발하는 원인이 되기도 한다[1]. 음료 및 음식을 통한 감염부터 상처를 통한 감염에 이르기까지 박테리아 감염은 1차대전 시기에 이르러서는 주요 사망의 원인으로 작용하였으나, 1928년 알렉산더 플레밍이 페니실린을 개발하여 이에 의한 사망률을 급격히 떨어지게 되었다[2]. 하지만 항생제 남용으로 인해 항생제 내성균의 발생이 증가하고 있는 추세이며, 최근 슈퍼박테리아로 알려진 다제항생제내성균의 확산은 유럽 및 미국을 비롯하여 국내에서도 커다란 문제로 인식되고 있다. 2016년 미국 질병관리본부 보고서에 따르면 미국을 기준으로 2013년도에만 200만명 이상이 항생제 내성균에 의해 감염되었으며 이 중 약 27,000명이 사망한 것으로 보고되었다[3]. 또한, 2014년 영국 수상 David Cameron 정부에 의해 작성된 ‘항생제 내성균에 대한 영국정부 보고서’에 의하면 항생제 내성균에 의한 감염은 지속적으로 증가하여 2050년 까지 누적 총 3억의 사망자(2014년~2050년)를 발생시키고, 이에 의한 사회적 손실 누적액은 \$100 trillion(약 113,636조)에 이를 것으로 예측한다[4] (그림 1.1). 한국의 경우 1일간 항생제 사용자 수는 1,000명당 34.8명으로 OECD 평균 21.1명보다 크게 웃도는 수치로 OECD 국가중 2위로 집계되어 항생제 사용으로 인한 부작용 및 위험성이 지속적으로 제기되고 있는 상황이다[5] (그림 1.2). 이런 항생제 남용은 항생제 내성 병원균 확산이라는 결과를 초래함으로써, 병원내 병원균 뿐 아니라, 환경 유래 박테리아에서 항생제 내성 활성이 발견되는 원인을 제공하게 되었다. 따라서 수술 뿐 아니라 일상생활에서의 상처에 의한 항생제 내성균 감염이 증가하고 있는 추세이다. 이러한 항생제 내성균 등에 의한 감염의 가장 커다란 문제는 초기증상은

경미하나 이후 급성 패혈증(혈액내 병원균 감염) 등으로 발전하여 응급 상황을 유발시키는 것이다[6].

최근 그램음성(gram negative) 항생제 내성균에 의한 감염이 빠른 속도로 증가하고 있는 실정으로 특히 ESBL(extended spectrum beta-lactamase)계 또는 CRE(carbapenem-resistant Enterobacteriaceae)계의 *Klebsiella pneumoniae* 와 *Escherichia coli* 가 전세계적으로 크게 유행하고 있다[7]. 이들은 광범위한 beta-lactam계 또는 carbapenem계 항생제에 항생제 내성을 가진 균주를 의미하며, 전체 *Klebsiella pneumoniae* 감염의 30%, *Escherichia coli* 감염의 20%, 정도가 ESBL 또는 CRE 관련 항생제 내성균으로 보고되고 있다[8]. ESBL 및 CRE 계열 항생제 내성 박테리아들은 cephalosporin, monobactam 및 최후의 항생제로 표현되는 carbapenem 등의 대부분의 beta-lactam계 항생제를 분해하는 것으로 알려져 있고, 이들 병원균으로부터 발전한 패혈증의 경우 사망률이 50% 이상으로 보고되고 있다[9]. 미국 질병관리본부 보고에 따르면 2017년 인도에서 미국으로 귀국한 70대 여성이 항생제내성균에 의해 감염되었으나, 이 환자로부터 분리된 항생제 내성균의 경우 미국에서 구입 가능한 26개 항생제 모두에 내성을 가지고 결과적으로 환자가 사망에 이르게 되었다[10]. 따라서 항생제 내성균 및 이에 의한 감염에 대한 관심이 증폭되고 있는 실정이다.

혈액 감염에 의해 발생하는 패혈증은 전 세계적으로 약 1800 만명 이상의 환자가 발생하며, 영유아 및 고령의 환자의 사망률은 70%에 이르는 무서운 질병이다. 일반인의 경우 패혈증에 의한 사망률은 30%에 이르며, 생존시에도 장기부전과 같은 다양한 부작용을 가지는 것으로 알려져 있다. 패혈증 치료에 있어 가장 커다란 문제는 초기 진단이 어려운 점이며, 이는 초기 패혈증 환자의 혈액에 존재하는 적은 농도의 병원균 수에 기인한다. 초기 패혈증의 경우 혈액 1mL에 약 1개에서 10개 미만의 병원균이 존재하는 것으로 알려져 있으며, 현존하는 병원균 검출

기술의 민감도 및 특이도의 한계로 인하여 검출에 어려움을 겪고 있는 현실이다[11]. 따라서, 부족한 민감도 문제를 해결하기 위하여, 환자의 시료 즉 혈액내 존재하는 병원균을 3~5일간 배양하고, 충분한 개체수의 병원균이 확보되면 병원균을 감별하고 이후 항생제 내성분석(antimicrobial susceptibility testing, AST)을 통하여 약제 특이성을 분석하는 것이 일반적이다[12]. 이러한 과정은 최종적으로 5~7일 정도 소요되며, 진단 분석의 긴 소요시간은 많은 경우에 있어 환자가 치료시기를 놓치게 되는 문제의 원인으로 작용한다. 따라서 패혈증이 의심되는 경우, 의사는 병원균의 진단 및 분석이 완료되기 전까지 수일간 다양한 종류의 항생제를 환자에게 투여하게 되며, 이는 발생한 항생제의 독성으로 환자의 장기 손상이 일어나고 의료비용의 증대로 이어진다. 따라서 혈액 감염의 조기치료를 통한 환자 생존율 증대를 위해, 혈액내 병원균의 빠르고 정확한 진단이 요구되고 있는 실정이다[13].

패혈증의 일반적인 진단법의 경우 앞서 언급한 바와 같이, 혈액 배양을 기반으로 하여 패혈증 균주를 확보하고, 배양된 균주의 패턴분석, PCR기반의 유전자분석이 주류를 이루고 있으나, 오랜 배양시간으로 인하여 진단에 요구되는 시간이 길어지는 문제점을 가지고 있다. 또한 염증표지 인자인 C반응 단백질(C-reactive protein, CRP) 및 프로칼시토닌(procalcitonin) 등이 패혈증 진단에 보편적으로 활용되고 있으나, 초기 패혈증의 경우 낮은 민감도로 인하여 초기 진단에 어려움을 겪고있다. 최근 패혈증 진단을 위한 신속 진단기술들이 다양한 기술적 경로를 통하여 개발되고 있으나, 현재 개발 초기 단계에 있거나, 민감도 정확도 면에서 초기 패혈증 진단에 어려움을 있는 상황이다.

본 연구의 궁극적인 목표는 혈액 등과 같이 복잡한 시료에 극 미량으로 존재하는 병원균의 신속 정확한 검출의 위한 분석 기술의 개발로써, 하나의 반응에서 다종의 병원균을 동시 검출 가능한 진단기술의 기반을 마련함에 있다. 이를 위하여

대표적인 다중 분석 기술인 Raman기술을 이용하고, 보다 높은 민감도를 확보하기 위하여 표면증강라만산란(Surface-Enhanced Raman Spectroscopy, SERS) 기술을 활용하였다. 또한 가장 넓이 사용되는 분자진단기술인 PCR기술을 SERS에 접목함으로써, PCR이 가지는 민감도와 Raman이 가지는 다중검출능을 모두 가지는 새로운 형태의 진단기술을 개발하고자 하였다.

PCR(Polymerase Chain Reaction)은 DNA의 원하는 부분을 복제, 증폭시키는 분자생물학적인 기술로서 사람의 게놈과 같은 매우 복잡하며 양이 지극히 미량인 DNA에서 연구자가 원하는 특정 DNA 단편만을 선택적으로 증폭시키는 기술로 증폭에 필요한 시간이 2시간 이하로 짧으며, 실험 과정이 단순하고, 전자동 기계로 증폭할 수 있기 때문에 최근 진단에 다양하게 사용되고 있다[14] (그림 1.3). 하지만 PCR은 형광 기반의 분석 기술로 시료당 하나 또는 두개 정도의 유전자 검출이 가능한 기술적 한계를 가지고 있다[15]. SERS는 나노입자의 표면처리를 통해 일반 Raman scattering에 비해 signal을 증강시키는 기술로 입사된 에너지가 다양한 nanogap을 갖는 금속 나노구조체로부터 증폭되어 방출되는 국소 표면 플라즈몬 공명 (Localized Surface Plasmon Resonance, LSPR) 효과가 일어나며 signal의 세기가 일반 Raman기술 대비 $10^4 \sim 10^6$ 배 증폭되어 특정 바이오 물질 및 화학 분자의 분석과 정량화가 이용된다[16, 17] (그림 1.4). 특히 금(Ag)이나 은(Au)과 같은 금속계열의 나노입자에서 이 같은 현상이 더욱 크게 나타나며, 사용되는 Raman dye별 고유의 Raman signal 패턴을 가지고 있어 다중 검출이 가능하다는 장점을 가지고 있다. 또한 Raman signal은 시간에 따라 줄지 않고, 온도 및 습도 등 주위 환경 영향에 민감하지 않아 다양한 환경에서 사용 가능하다는 장점을 가지고 있다[18]. 하지만 Raman 기술의 문제는 재현성에 있다는 점이다. 비록 SERS가 기존 Raman 분석법 대비 높은 민감도를 가지고 있는 것으로 알려져 있으나,

질량분석법이나 PCR법과 같이 높은 민감도를 가지는 분석법들과 비교하여 상대적으로 낮은 분석 민감도 및 특이도를 가지며 이로 인한 반복 재현성이 부족하다는 문제가 제기되고 있다. 따라서 본 연구에서는 PCR과 SERS 두 기술을 통합시킴으로써 이들의 민감도 및 다중 분석이라는 장점을 모두 가지며, 동시에 각각의 약점인 다중분석능과 민감도를 극복하고자 하였다.

2. 시약 및 방법

본 연구에서는 *Klebsiella pneumoniae*의 검출을 목표로 하여 *Klebsiella pneumoniae* (KCTC 2208)를 nutrient broth 배지로 하여 35°C, 180RPM 조건에서 배양하였다. Nutrient broth 5mL와 *Klebsiella pneumoniae* 50uL를 따서 12시간 동안 seed culture하고 seed culture 용액 200uL와 nutrient broth 5mL를 따서 5시간 동안 main culture 하였다.

2.1 *Klebsiella pneumoniae* DNA의 추출

실험에 필요한 *Klebsiella pneumoniae*의 DNA를 얻기 위하여 균에서 DNA를 추출하였다. 5시간 동안 main culture한 균을 Nanodrop2000c spectrophotometer (Thermo fisher)를 이용하여 OD600 값을 측정하고 이를 토대로 균의 개수가 10^6 개가 되도록 1.5mL centrifuge tube에 희석하였다. Buffer ATL 100uL와 proteinase K 20uL를 넣어주고 15초 동안 vortexing 한 후, 56°C에서 15분마다 5초씩 섞어주면서 2시간동안 incubation 하였다. Buffer AL 200uL를 넣고 15초간 vortexing 하고 70°C 에서 10분간 보관 후 15초동안 vortexing 하였다. Ethanol (96-100%) 200uL를 넣고 15초간 vortexing 하고 본 결과물을 spin column에 옮긴 후 8000RPM에서 1분간 원심분리 하였다. Spin column을 새로운 2mL collection tube에 옮기고 Buffer AW2 500uL를 넣고 20000 g에서 3분간 원심분리 하였다. Collection tube를 빼고 spin column을 새로운 1.5mL centrifuge

tube에 넣고 Buffer AE 200uL 첨가한 후 1분간 상온에서 보관 후 8000RPM에서 1분간 원심분리 하였다. 분리하여 나온 용액이 *Klebsiella pneumoniae*의 DNA로 blank를 buffer AE 로 하여 Nanodrop을 이용하여 DNA 농도를 측정하였고 이때 DNA 한 가닥은 5.17 fg으로 계산하였다.

2.2 *Klebsiella pneumoniae* 특이적 유전자 증폭

병원균 특이적 유전자의 단일 DNA를 증폭시키기 위하여 asymmetric PCR을 수행하였다. Forward primer와 Reverse primer의 비를 각각 100:1, 50:1, 10:1, 1:1로 하여 넣고, target을 넣은 후 나머지 용액은 D.W.를 넣어 총 50uL를 맞추어 PCR 하였다. 병원균의 농도를 10⁶개부터 0개까지 넣어주고 95°C로 10분간 PCR Master mix를 활성화 후 95°C 30초로 Denaturation, 59°C 30초로 Annealing, 72°C 1분간 extension을 총 80번 반복하여 증폭하였다. 증폭이 끝난 뒤 72°C에서 5분간 incubation 하였다. Forward primer는 ‘GAC CGC TGA GTA ATG TCT GG’ 이고, reverse primer 는 TGC GGT ATT AGC TAC CGT T, Target 은 GAC GGG TGA GTA ATG TCT GGG AAA CTG CCT GAT GGA GGG GGA TAA CTA CTG GAA ACG GTA GCT AAT ACC GCA’이다(표 2.1).

2.3. Particle 표면의 Silica coating[54]

Particle 1nM에 MPTMS 0.1mM을 24시간동안 1400RPM에서 섞어준다. pH 7.4, 10mM의 Sodium phosphate 용액을 만들어 준 뒤 이 용액을 particle 용액에 넣어주고 13,200RPM에서 30분동안 centrifuge 하였다. 상층액을 제거하고 sodium phosphate에 다시 분산시킨 후 다시 centrifuge 하여 상층액을 제거하고 ultra-pure water에 분산시켜 주었다.

2.4. Forward & reverse primer, synthetic target, hairpin structure DNA 디자인

Forward primer와 reverse primer는 National Center for Biotechnology Information primer BLAST 툴을 이용하여 제작하고 해당 primer에 대한 annealing temperature는 59°C 로 gradient PCR을 통해 알아내었다. Forward primer는 5'-GAC GGG TGA GTA ATG TCT GG-3'이고 reverse primer는 5'-TGC GGT ATT AGC TAC CGT T-3'이다. 해당 target은 Blast Local Alignment Search Tool (BLAST)를 이용하여 *Klebsiella pneumoniae*의 유전자를 알아내었다. 알아낸 유전자를 바탕으로 target sequence를 정하고 hairpin structure DNA는 Integrated DNA Technologies tool을 이용하여 디자인하였다. Target sequence는 5'-GAC GGG TGA GTA ATG TCT GGG AAA CTG CCT GAT GGA GGG GGA TAA CTA CTG GAA ACG GTA GCT AAT ACC GCA-3' 이다. Hairpin structure DNA는 [Thiol][18atom spacer]CCC TCC TGT ACA AGT TGA AAC TGC CTG ATG GAG GG[FAM]으로 양 끝에 thiol기 와 Raman dye 종류 중 하나인 FAM이 부착되어 있다. Hairpin structure DNA의 접힌 hairpin 구조의 binding temperature 값은 50.5°C 로 그 이상의 온도로 높여주면 hairpin structure가 풀린다. 이에 일부 상보적인 sequence를 가지는 Blocking DNA의 sequence는 TAG TTA TCC CCC TCC ATC AGG CAG TTT C이다(표 2.1).

2.5. SERS probe 제작

Hairpin structure DNA를 앞서 합성한 particle에 부착하기 위하여 thiol기의 활성화를 하는데, 0.1 M DTT 50 uL와 100µM DNA 50uL를 넣어 1시간 동안 반응하였다. 반응 후 NAP-5-Column을 이용하여 분리 정제 후 thiol기가 활성화된 DNA를 Nanodrop2000c 을 이용하여 DNA 양을 측정하였다. 10µM DNA를 100uL 따서 0.1nM의 silver-coated gold nano star particle 10mL에 넣어주었다. 이 때 버퍼용액은 0.01mM PB in 0.01% TWEEN20이고 0.01mM PB in 0.01% TWEEN20, 2M NaCl 용액을 이용하여 salt aging

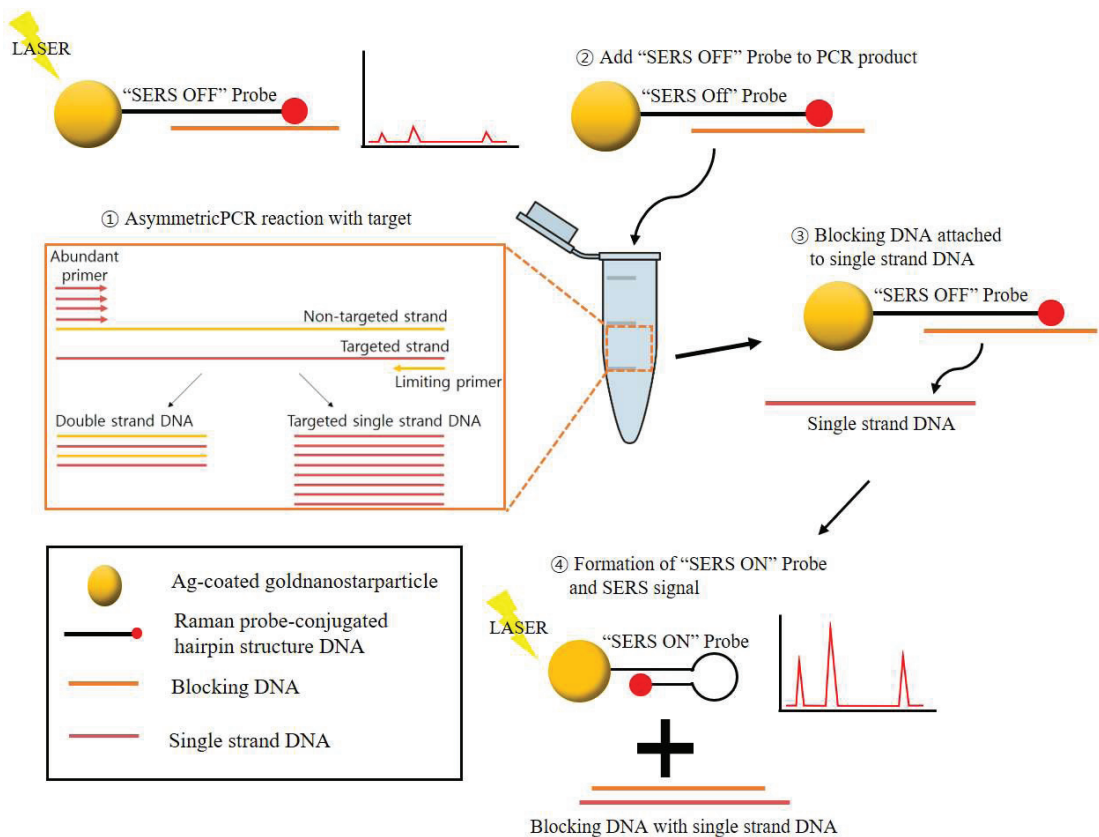
하였다. 이때 NaCl의 농도는 0.05M씩 증가되도록 NaCl을 넣어주고 10초간 sonication, 20분간 incubation 하는 과정을 NaCl이 300mM이 되도록 반복 후 반응이 끝난 뒤 overnight 하였다. Particle을 Amicon® Ultra Centrifugal Filters에 넣고 3분동안 6000 RPM으로 원심 분리하여 particle에 고정되지 않고 시료에 남아있는 hairpin structure DNA를 제거하였다. Washing buffer는 증류수를 이용하여 3번 원심분리 하였다. Particle 농도는 0.1nM로 하고 target으로 인하여 “SERS OFF” probe에서 “SERS ON” probe로 전환되기 위해서는 blocking DNA의 부착이 필요해 0.1nM “SERS ON” probe에 0.1μM blocking DNA를 넣고 48°C에서 12시간 incubation 하였다. Blocking DNA의 sequence는 TAG TTA TCC CCC TCC ATC AGG CAG TTT C이다. 남은 blocking DNA는 10분동안 7000RPM에서 centrifuge를 통해 제거하고 제작한 “SERS OFF” probe는 target과 48°C에서 1시간 incubation 하고 그 시료의 SERS 측정, target을 검출한다. SERS는 Raman spectroscopy를 이용하여 측정하고 laser의 파장은 532nm, objective lens는 20배율을 사용하였다. Room temperature에서 0.5초씩 3번 accumulation 하여 측정하였다. 샘플은 모세관에 10uL를 넣어 측정하고 baseline 설정은 Nanoscope software 내에 있는 baseline correction을 이용하였다.

3. 결과 및 고찰

3.1. 다중검출 기술개발을 위한 이론적 배경

본 검출 실험에서는 SERS 기반의 probe를 제작하고 이를 통하여 *Klebsiella pneumoniae*를 검출하였다(Scheme 2.1). 우선 abundant primer와 limiting primer를 넣어 asymmetric PCR을 진행하여 single strand DNA를 증폭하였다 (Scheme 2.1 ①). 증폭된 DNA 들을 “SERS OFF” probe에 넣고 1시간, 48°C에서 반응하였다(Scheme 2.1 ②). 반응하는 동안 single strand DNA가 “SERS OFF” probe에 부착되어 있는 blocking DNA와 결합한다(Scheme 2.1 ③). Blocking DNA가 hairpin structure로부터 떨어져 곧게 펴져 있는 hairpin structure DNA가 구부러져 “SERS ON” probe를 형성한다(Scheme 2.1 ④). 적은 농도의 target도 검출하기 위하여 PCR을 하는데 primer의 양이 다른 asymmetric PCR해준다. 그 이유는 일반 PCR의 경우 double strand DNA만 증폭되지만 asymmetric PCR은 일반 PCR과 달리 넣어주는 forward primer와 reverse primer의 양이 달라 double strand DNA 뿐 아니라 single strand DNA를 형성한다(Scheme 2.2). 원하는 single strand DNA 형성에 해당하는 primer를 abundant primer 라 부르고, 제한된 증폭을 하는 primer를 limiting primer라 하는데 본 실험에서 abundant primer는 forward primer이고 limiting primer는 reverse primer이다. Scheme 2.1 ③에 해당하는 과정인 “SERS OFF” Probe에서 blocking DNA를 떼어내는 과정에서 double strand DNA보다 single strand DNA가 훨씬 효과적인데 그 이유는 single strand DNA에 해당하는 상보적 염기서열이 없어 blocking DNA와의 결합에 유리하기 때문이다. 증폭된 single strand DNA는 “SERS OFF” probe에 고정된 blocking DNA와 반응하기 위하여 “SERS OFF” probe를 제작, scheme 2.3의 반응을 진행하였다. Hairpin structure DNA는 총 6개의 상보적 base pairs 로 인하여 hairpin bend를 형성하는데 이를 막기 위하여 합성한 “SERS ON” probe 에 blocking DNA를 넣고 12시간동안 48°C 로

incubation 하였다. 그 이유는 hairpin bend의 binding temperature가 50.5°C 로 hairpin structure가 열려야 blocking DNA와 결합하기 때문이다(그림 2.9 a). 12시간, 48°C incubation을 통하여 시료에 충분한 열을 가해 hairpin structure bend 결합이 풀리게 되고, hairpin structure DNA와 blocking DNA가 가장 잘 결합할 수 있는 온도로 잘 결합한다. Hairpin structure DNA와 결합한 blocking DNA의 binding temperature는 55.7°C로 hairpin bend binding temperature 인 50.5°C 보다 5.2°C 높아 경쟁에 유리하다(그림 2.9 b). 따라서 blocking DNA가 hairpin structure DNA에 결합되고 이는 Raman dye가 particle 으로부터 멀어지게 되면서 SERS signal이 줄어든다. 이는 Raman dye와 particle간의 거리에 따라 SERS signal이 바뀌기 때문으로 거리가 가까우면 SERS signal이 증가하고 거리가 멀어질수록 SERS signal이 줄어들기 때문이다[56] (그림 2.10). 제작한 “SERS OFF” probe에 asymmetric PCR을 통해 얻은 single strand DNA를 넣고 1시간, 48°C로 incubation 한다. “SERS OFF” probe에 결합되어있던 blocking DNA가 single strand DNA와 결합하게 되어 떨어져 나가고 hairpin structure DNA는 다시 구부러진다(Scheme 2.4). 이는 다시 particle과 Raman dye의 거리를 줄어들게 하여 강한 SERS signal을 내게 한다. 1시간, 48°C의 incubation 시간과 온도는 blocking DNA와 single strand DNA가 결합하게 하고 만약 single strand DNA가 없는 경우 hairpin structure DNA와 blocking DNA의 결합을 유지시킨다. 이때 single strand DNA가 있을 경우 blocking DNA가 hairpin structure DNA에 결합하지 않고 single strand DNA에 결합하는 이유는 서로 다른 binding temperature 때문이다 (Scheme 2.5). Blocking DNA와 hairpin structure DNA의 binding temperature는 55.7°C, blocking DNA와 single strand DNA의 binding temperature는 61.2°C 로 blocking DNA와 single strand DNA의 결합이 blocking DNA와 hairpin structure DNA간의 결합보다 안정하다는 것을 의미하고 따라서 single strand DNA가 존재할 경우 blocking



DNA와의 결합에 hairpin structure DNA보다 우위를 갖고, blocking DNA의 migration이 일어나 *Klebsiella pneumoniae*의 검출이 가능하다. 따라서 본 이론을 실제 실험에 적용하여 데이터를 얻고 증명하였다.

그림 1. 헤어핀 기반의 "SERS" probe 를 이용한 전반적 *Klebsiella pneumoniae* 검출단계 모식도

3.2. "SERS" Probe 를 위한 nanoparticle 조건 확인

PCR 후 증폭된 target을 검출하기 위해 "SERS" probe를 합성, 제작하였다. SERS 를 위하여 nano particle 4종류를 합성하고 hairpin structure DNA를 부착하여 SERS signal를 측정하였다. 실험실에서 합성하고 사용한 nanoparticle은 총 4가지 종류로

gold nanoparticle, gold nanostar particle, silver nanostar particle, silver-coated gold nanostar particle이고 각 particle 합성 후 TEM을 통하여 잘 합성되었는지 확인하였다. (그림 2.17) (a)는 gold nanoparticle, (b)는 gold nanostar particle, (c)는 silver starparticle, (d)는 silver-coated gold nanostar particle (e)는 gold nanoparticle 기반의 “SERS” probe 의 SERS signal, (f)는 gold nanostar particle 기반의 “SERS” probe 의 SERS signal, (g)는 silver starparticle 기반의 “SERS” probe의 SERS signal, (h)는 silver-coated gold nanostar particle 기반의 “SERS” probe 의 SERS signal이다. (d) Silver coated gold nanostar particle 경우에는 안쪽에 더 진한 색상으로 star 모양을 확인할 수 있는데 이는 gold nanostar가 있다는 것을 의미하고 silver는 회색을 띄며 균일하게 코팅 된 것을 알 수 있다. SERS signal를 보면 (h) 가 가장 높는데 이는 particle의 특성에 있다. Gold nanoparticle 보다 silver nanoparticle 일 때 SERS signal이 좋고[61] 구형보다 표면이 뾰족할 때 SERS signal이 좋다.[62] 따라서 두가지 특징을 모두 가지고 있는 silver-coated gold nanostar particle의 SERS signal이 가장 좋게 나타나 본 particle을 “SERS” probe로 사용하였다.

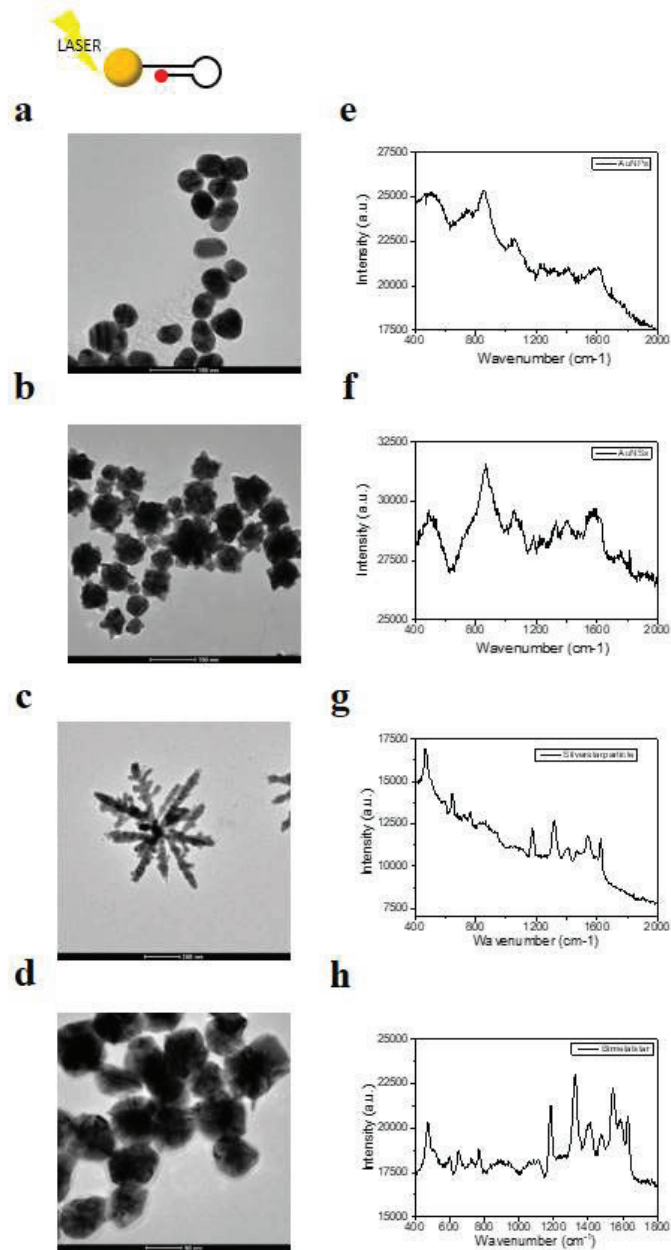


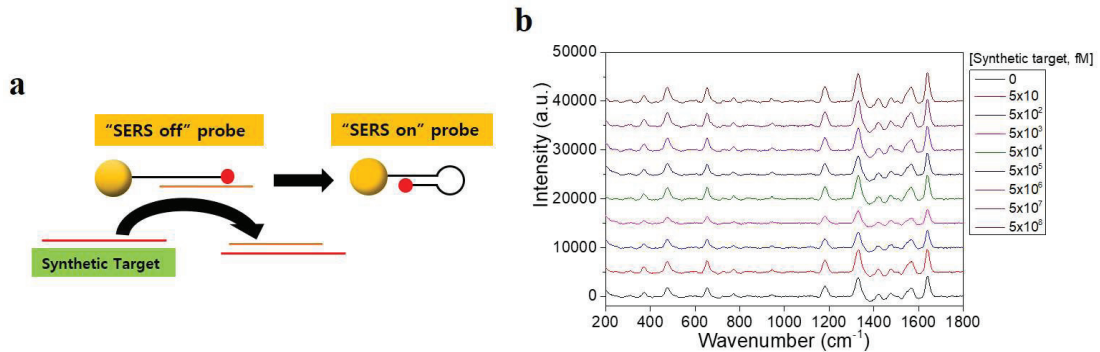
그림2. Nanoparticle TEM image 와 SERS signal (a) 60nm gold nanoparticle, (b) 90nm gold nanostar particle, (c) silver starparticle, (d) silver-coated gold nanostar particle (e) gold nanoparticle SERS signal, (f) gold nanostar particle SERS signal, (g) silver starparticle SERS signal, (h) silver-coated gold nanostar particle SERS signal

3.3 “SERS” probe 조건 확인

“SERS” probe 합성 후 농도별로 SERS를 찍어 FAM의 SERS signal 파장대와 signal를 확인하고 총 6개의 대표적 파장대를 파악하였다(그림 2.18). 6개의 파장대는 476 cm^{-1} , 653 cm^{-1} , 1180 cm^{-1} , 1329 cm^{-1} , 1639 cm^{-1} 로 농도에 비례하여 증가하는 것을 확인하였다. Particle 한 개당 고정된 DNA 개수에 따른 SERS를 측정하여 비교하였다. 고정된 DNA 개수는 각각 2076, 1557, 1038, 519, 207 개로 1038개의 DNA가 고정되었을 때 가장 높은 SERS signal를 보였다(그림 2.19 a). 1329 cm^{-1} 파장대에서의 signal 값을 비교하였을 때 너무 적거나 많이 고정시키면 signal이 가장 낮고 중간값인 1038개일때 가장 높는데 이는 고정된 DNA 개수가 너무 많으면 표면당 DNA의 밀도가 높아 hairpin structure가 구부러지기 더 어려울 것이라 예상된다[63](그림 2.19 b). 따라서 particle 한 개당 고정한 DNA 수는 1038개로 하였다. SERS signal 최적화를 위하여 buffer의 조건을 PB 0.01M in 0.01% TWEEN 20과 PBS 0.01M in 0.01% TWEEN 20으로 하여 실험하였다(그림 2.20). 0.01M PBS in 0.01% TWEEN20의 경우 sodium이 150mM 들어있어 blocking DNA가 hairpin structure DNA에 고정되기 더 유리한 환경에 있다. 그 이유는 particle에 모여 있는 DNA는 -극을 띠고 있어 blocking DNA가 가까이 가기 힘들지만 sodium이 있으면 이온 상태로 있어 -극을 중화시켜 blocking DNA가 hairpin structure DNA와의 결합을 유리하게 한다. 따라서 0.01M PBS in 0.01% TWEEN20 buffer 일 때 “SERS OFF” probe와 “SERS ON” probe의 차이가 더 크다(그림 2.20 b). 각 시료를 incubation 후 SERS 측정 전 sonication을 하여 signal의 변화를 보았다. “SERS ON” probe의 경우 sonication을 하고 난 뒤의 signal이 더 증가하였고 “SERS OFF” probe의 경우 sonication을 한 시료의 signal이 더 감소하였다(그림 2.21). 그 이유는 incubation 시간 동안 48°C 의 온도로 있기 때문에 particle의 뭉침 현상이 일어나고 정확하지 않은 signal의 발생으로

이어진다. 하지만 sonication을 하게 되면 particle의 뭉침 현상이 풀려 더 정확한 SERS signal이 나오게 된다. 그 이유는 particle이 뭉쳐 있을 경우 사이에 낀 Raman dye의 영향이 있을 수 있기 때문이다. “SERS” probe의 온도안정성을 확인하기 위하여 48°C - 60°C 로 12 시간 incubation 하였다. Incubation 하지 않은 “SERS ON” probe와 비교하였을 때 incubation 온도가 48°C 인 것이 가장 차이가 없었고 온도가 점점 증가할 수록 signal이 점차 감소하였다(그림 2.22). 이는 온도가 증가할 수록 고정된 hairpin structure DNA가 thiol 결합을 끊고 탈락하기 때문에 incubation 온도는 48°C 로 설정하였다[64]. “SERS ON” probe에 blocking DNA를 넣고 48°C 로 12시간 incubation 하게 되면 hairpin structure DNA가 열리게 되면서 blocking DNA가 결합한다(그림 2.23 a). Blocking DNA를 100nM, 80nM, 60nM, 40nM, 20nM 농도로 넣고 incubation 하여 실제 SERS signal을 확인하였더니 blocking DNA의 농도가 커질수록 SERS signal이 감소하였다(그림 2.23 b). 파장별 막대그래프로 추세를 비교하였더니 확연히 감소하는 것을 확인, 100nM에서 가장 작은 signal를 보였다(그림 2.25 a). 그 중에서 확연한 감소를 보인 478 cm^{-1} 데이터 값을 이용, standard curve를 계산하여 R^2 값이 0.9993로 비례적으로 감소하는 것을 확인하였다(그림 2.25 b). 다음으로 synthetic target을 농도별로 넣어 blocking DNA와의 결합을 통해 “SERS ON” probe를 형성하는 것을 보았다(그림 2.26 a). Synthetic target의 농도를 5×10^8 , 5×10^7 , 5×10^6 , 5×10^5 , 5×10^4 , 5×10^3 , 5×10^2 , 5×10^1 로 하여 48°C 1시간동안 incubation 하고 SERS signal 변화 확인, 농도가 증가될수록 SERS signal이 증가하였다(그림 2.26 b). 파장대별로 확인을 하면 각 파장별로 농도가 증가될 때마다 SERS signal이 증가하였다(그림 2.27). 앞에서 했던 476 cm^{-1} 파장대에서 standard curve를 계산하여 R^2 값이 0.92318로 비례적으로 증가하는 것을 확인하였다(그림 2.28 b). 마지막으로 특이적 결합을 확인하기 위하여 “SERS OFF” probe에 scramble DNA 5종을 넣어 48°C로 1시간 incubation 하였다.

사용된 scramble DNA 5종은 CAA TTC ATG GAC CAA GC, CCG TCC CAA GTC ATG AAA C, AGC ACA GAG AGC TTG, TGC GGG ACT TAA CCC AAC A, CTC TCA ATC TGT CAA



TCC TTA TT이다(표 2.2). 그 결과 synthetic target을 넣고 반응한 것은 positive control과 비슷한 signal를 보였고 scramble DNA 5종은 negative control보다 조금 높은 signal를 보였다(그림 2.29). 각 파장대를 막대그래프로 정리하여 비교하였더니 더 확연한 차이가 나타났다(그림 2.30). 이는 blocking DNA와 상보적인 서열을 가지는 synthetic target의 경우 blocking DNA와 결합하여 hairpin structure DNA에서 떨어져 나가 “SERS ON” probe를 형성하고 scramble DNA 5종은 blocking DNA와 상보적이지 않아 결합하지 않고 hairpin structure DNA에 결합되어 있어 negative control과 비슷한 결과가 나온 것이다.

그림 3. Synthetic target에 blocking DNA의 결합 및 농도에 따른 SERS signal 증가 추이
(a) Synthetic target과 blocking DNA의 결합으로 인한 “SERS OFF” probe 에서 “SERS ON” 변환 도식, (b) SERS Profile

3.4. “SERS” probe 를 이용한 gDNA 검출 조건 확인

“SERS OFF” probe와 실제 *Klebsiella pneumoniae*의 asymmetric PCR을 통해 증폭한 결과물의 검출 특이성과 민감도를 확인하기 위하여 5종의 균인 *Klebsiella pneumoniae*,

Escherichia coli, *Staphylococcus aureus*, *Candida albican*, *Salmonella enterica*에 대한 PCR 조건인 annealing temperature를 잡았다. 그 이유는 조건을 통해 PCR을 하고 각 균의 single strand DNA를 얻어 특이성실험을 진행해야 하기 때문이다. 실험결과 58°C의 온도에서 PCR이 일어났고 각 target primer가 특이적으로 해당 target만을 증폭시키는 것을 확인하였다(그림 2.31 a-d, 표 2.3). 확인한 조건에 forward primer: reverse primer 양을 100:1로 넣고 PCR 하여 각 균의 single strand DNA를 증폭하고 “SERS OFF” probe에 넣고 1시간 48°C로 incubation 하였다. 그 이유는 특이적 검출을 위해서인데, *Klebsiella pneumoniae* 외 다른 균의 single strand DNA와 반응하면 probe로서의 역할을 하지 못하기 때문이다. 실험결과 negative control과 비교하여 P-value (* $p < 0.05$, ** $p < 0.001$, and *** $p < 0.001$ versus control)를 측정, *Klebsiella pneumoniae*는 ***, *Escherichia coli*는 **, *Candida albican*는 **, *Staphylococcus aureus*는 ** 값을 얻었다(그림 2.32). 따라서 5종의 균에 대해 특이성을 가지는 것을 확인하였다. 다음으로 *Klebsiella pneumoniae* 검출 한계를 위해 target의 개수를 10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10, 5, 1, 0개로 하여 PCR 후 “SERS OFF” probe에 1시간 48°C로 incubation 하였다. 이때 forward primer: reverse primer 의 비율은 100:1로 하였다. 실험결과 10^6 개부터 1개까지 모두 검출하였다. 따라서 검출 한계는 gDNA 1개 수준임을 확인하였다(그림 4).

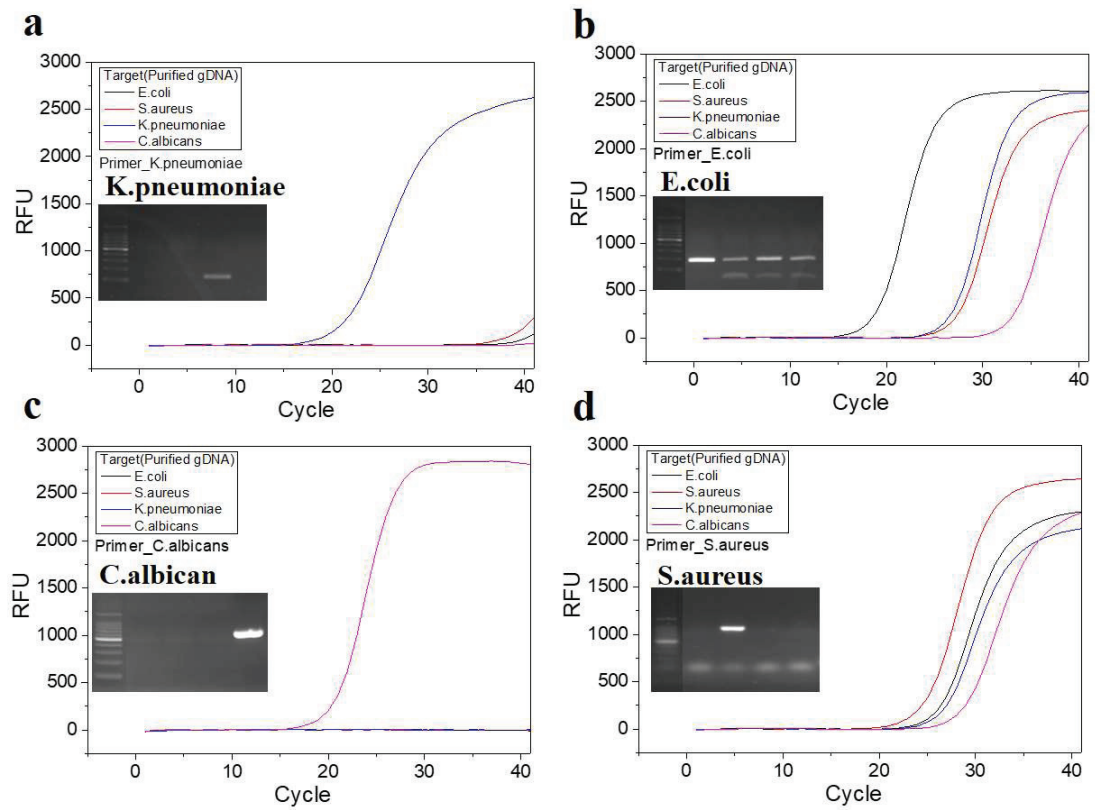


그림 4. *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus* PCR 결과

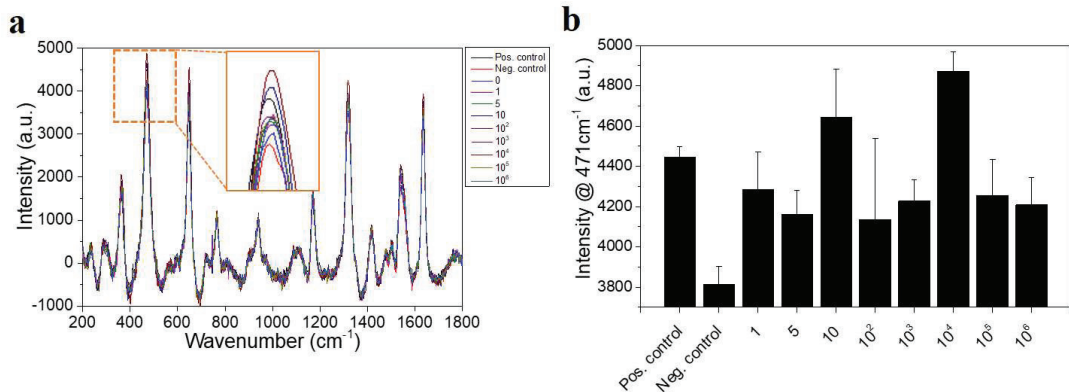


그림 5. 민감성검사를 위한 *Klebsiella pneumoniae* target 개수에 따른 asymmetric PCR 후 “SERS OFF” probe 와 1 시간 48°C로 incubation 후 SERS signal 비교 (a) SERS profile, (b) Wavenumber 471cm⁻¹ 에서의 SERS signal

4. 결론

본 연구에서는 하나의 반응에서 다종의 병원성유전자를 빠르고 정확한 분석을 위한 기반기술을 개발하기 위하여, 표준화된 분자진단 기술인 PCR법에 Raman 분석법을 도입함으로써 새로운 형태의 분석기술을 개발하였다. 연구의 최종 목표는 PCR이 가지고 있는 태생적 문제인 다중 분석에 있어, 복잡한 광학시스템을 요구로 하며 이로 인하여 하나의 반응에서 일반적으로 두개의 특이적 유전자만 분석 가능한 한계를 극복한 새로운 형태의 PCR기술의 개발이다.

이를 위하여 다중 분석에 장점을 가진 것으로 알려진 Raman 분광법을 이용하여 증폭된 유전자의 분석법을 제시하였다. Raman 분광법 기반의 분석방법은 Raman dye 종류에 따라 다양한 형태의 Raman signal 패턴이 발생하고, 분석하고자 하는 target에 따라 특정 Raman dye가 부착된 DNA 표지 인자 즉 “SERS” probe를 설계하며, target-“SERS” probe 세트에 따라 각각의 Raman dye가 반응함으로써 target 특이적 Raman

signal 패턴을 발생시키는 원리이다. PCR과의 연계를 위하여 병원균 특이적 primer를 설계하고, 이에 의해 증폭된 병원균 특이적 증폭유전자가 Raman dye가 고정된 “SERS” probe와 반응하고 이에 의해 Raman signal이 증폭되도록 실험을 설계하였다. 또한 Raman signal의 극대화를 위하여 silver-coated gold nano star particle기반의 “SERS” probe를 제작하였다. 보다 구체적으로, hairpin structure DNA의 3’말단에 Raman dye를 고정하고 5’말단을 금속 나노입자에 고정화 시킴으로써 SERS를 유도하였으며, hairpin structure DNA의 일부 핵산 염기 서열과 상보적인 염기서열을 가지는 blocking DNA를 제작하여 구조가 펼쳐짐으로써 병원균 특이적 증폭유전자가 없는 환경에서는 SERS signal이 나오지 않는 형태의 센서를 제작하였다. Gold nanoparticle, gold nanostar particle, silver nanostar particle, silver-coated gold nanostar particle등을 제작하고, 표면에 Raman dye가 달린 hairpin structure DNA를 고정시켜 센서로서의 SERS 극대화 조건을 확립하였다.

본 연구에서는 패혈증 관련 감염성 병원균을 검출 target 인자로 선정하여 연구를 진행하였는데, 이는 사망률 30%에 이르는 패혈증의 경우 응급실내 환자사망원인의 가장 커다란 비중을 차지하고 있기 때문이다. 특히, 3세 미만의 어린이나 70이상 노인의 경우 생존율이 20% 미만으로 알려져 감염 초기 빠른 진단을 통해 정확한 치료가 요구되는 주요 질병인 패혈증에 있어 *E. coli*, *S. aureus* 등과 함께 가장 높은 비율로 발생하는 *Klebsiella pneumoniae*를 검출 target으로 선정하였다. 따라서, *Klebsiella pneumoniae* 특이적 유전자 증폭을 위한 primer를 설계하고 gradient PCR을 통하여 최적의 PCR조건을 확보하였다. 또한 제작한 primer가 *E. coli*, *S. aureus*, *C. albicans* 등과 같은 다른 종의 균주의 유전자 증폭에 관여하지 않음을 확인함으로써 병원균 특이적 유전자 증폭이 가능함을 확인하였다.

다음으로, 특이적으로 증폭된 *Klebsiella pneumoniae*의 증폭된 DNA와 반응하는

blocking DNA의 경우 이중나선구조 DNA가 아닌 단일 가닥 DNA임에 착안하여, 5'-3' 방향의 DNA만을 선별적으로 증폭시키는 asymmetric PCR 조건을 확립하였다. 이를 통해 증폭된 single strand DNA가 앞서 제작한 “SERS” probe와 특이적 반응을 통하여 SERS를 형성하는 것을 확인하기 위하여 *E. coli*, *S. aureus*, *C. albicans*, *S. enterica* 4종의 음성대조균 병원성 유전자를 각각의 특이적 primer를 이용하여 증폭시키고 증폭된 DNA를 *Klebsiella pneumoniae*-targeting “SERS” probe와 반응 시킴으로써 비특이적 SERS 증폭을 확인하였다. 실험 결과 *Klebsiella pneumoniae* 특이적 증폭 유전자만 SERS를 증폭시키는 것을 확인함으로써, 본 연구를 통하여 제작한 “SERS” probe가 병원균 특이적으로 SERS를 증폭시킴을 확인하였다. 마지막으로 개발한 분석기술의 민감도를 확인하기 위하여 *Klebsiella pneumoniae*로부터 분리된 gDNA를 serial dilution하여 병원균의 농도를 10⁶부터 1개까지 세분화한 가상의 시료를 준비하였다. 이후 이를 *Klebsiella pneumoniae* 특이적 primer를 이용하여 증폭시킨 후 반응 한계를 조사한 결과 하나 정도의 박테리아로부터 분리된 gDNA로부터 증폭된 시료에서도 *Klebsiella pneumoniae* 특이적 SERS가 증가됨을 확인할 수 있었다. 이들 결과는 생체시료로부터 병원균을 단일세포 수준으로 분리하고, 이의 유전자의 확보 및 증폭 가능한 환경에서 본 검출기술의 검출한계 농도가 1cfu에 이를 수 있음을 보여주는 결과이다.

본 연구에서 개발한 기술은 PCR기반의 병원균 특이적 유전자 증폭과 이의 검출을 위한 “SERS” probe를 통합시킴으로써, 향후 다양한 감염성 병원균을 하나의 시료, 하나의 반응체로부터 동시 검출 가능한 기술의 기반기술로 활용 가능할 것으로 기대된다. 향후 추가 연구를 통하여 다양한 병원균 특이적 “SERS” probe를 제작하고, 각각의 “SERS” probe별 다른 Raman dye를 이용함으로써 다중 분석, 다중 검출 기술로서의 발전이 가능할 것으로 기대된다.

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2024 기초과학연구소보

Degree of simultaneous approximation of multivariate functions by neural networks with one hidden layer^{*}

N.W. Hahm

Abstract. Many authors([1], [4], [5], [6]) have been examined simultaneous approximation by neural networks with one hidden layer. Hahm and Hong ([5], [6]) suggested some simultaneous approximation results for univariate functions. Hahm and Hong [7] obtained degree of multivariate functions by generalized translation networks with one hidden layer. In this paper, we suggest degree of simultaneous approximation of multivariate function by neural networks with one hidden layer.

AMS Subject Classification (2020): 65D15, 41A25

Keywords: Simultaneous approximation, Degree of approximation, Neural networks, Bernstein polynomials

1. Introduction and Preliminaries

Recently, many researchers([1], [3], [7]) investigated the density and the complexity problems of multivariate functions by neural networks with one hidden layer.

In [5], [6], Hahm and Hong suggested some results related to the simultaneous approximation by neural networks with one hidden layer but the target functions were univariate. Using Bernstein polynomials and the modulus of continuity, we suggest degree of simultaneous approximation of multivariate function by neural networks with one hidden layer in this paper.

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THE NUMBER OF ZEROS OF LINEAR COMBINATIONS OF L -FUNCTIONS NEAR THE CRITICAL LINE*

By

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Abstract. In this paper, we investigate the zeros near the critical line of linear combinations of L -functions belonging to a large class, which conjecturally contains all L -functions arising from automorphic representations on $\mathrm{GL}(n)$. More precisely, if $L_1, \dots, L_J$ are distinct primitive L -functions with $J \geq 2$, and b_j are any non-zero real numbers, we prove that the number of zeros of $F(s) = \sum_{j=1}^J b_j L_j(s)$ in the region $\mathrm{Re}(s) \geq 1/2 + 1/G(T)$ and $\mathrm{Im}(s) \in [T, 2T]$ is asymptotic to $K_0 TG(T)/\sqrt{\log G(T)}$ uniformly in the range $\log \log T \leq G(T) \leq (\log T)^\nu$, where K_0 is a certain positive constant that depends on J and the L_j . This establishes a generalization of a conjecture of Hejhal in this range. Moreover, the exponent ν verifies $\nu \asymp 1/J$ as J grows.

1 Introduction

The theory of L -functions has become a central part of modern number theory, due to its connection to various arithmetic, geometric and algebraic objects. L -functions are represented by Dirichlet series which are absolutely convergent in half-planes. They satisfy certain conditions, including having a meromorphic continuation, an Euler product over primes and a functional equation. The prototypical example of an L -function is the Riemann zeta function. Other important examples include Dirichlet L -functions attached to primitive Dirichlet characters, and the Hasse–Weil L -functions attached to elliptic curves. The Langlands program predicts that all L -functions arise from automorphic representations over $\mathrm{GL}(n)$.

L -functions are predicted to verify several hypotheses, the most important of which is the generalized Riemann hypothesis (GRH), which asserts that all non-trivial zeros of L -functions lie on the critical line $\mathrm{Re}(s) = 1/2$. On the other hand, there exist various Dirichlet series which have arithmetical significance and satisfy a functional equation, but are not L -functions, since they do not possess an

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Discrepancy bounds for the distribution of L -functions near the critical line

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Abstract

We investigate the joint distribution of L -functions on the line $\sigma = 1/2 + 1/G(T)$ and $t \in [T, 2T]$, where $\log \log T \leq G(T) \leq \log T/(\log \log T)^2$. We obtain an upper bound on the discrepancy between the joint distribution of L -functions and that of their random models. As an application we prove an asymptotic expansion of a multi-dimensional version of Selberg's central limit theorem for L -functions on $\sigma = 1/2 + 1/G(T)$ and $t \in [T, 2T]$, where $(\log T)^\varepsilon \leq G(T) \leq \log T/(\log \log T)^{2+\varepsilon}$ for $\varepsilon > 0$.

2010 Mathematics Subject Classification: 11M41 (Primary); 11M06, 11M26 (Secondary)

1. Introduction

We investigate the distribution of the Riemann zeta function $\zeta(s)$ for $\operatorname{Re}(s) > 1/2$ using its probabilistic model defined by the random Euler product

$$\zeta(\sigma, \mathbb{X}) = \prod_p \left(1 - \frac{\mathbb{X}(p)}{p^\sigma} \right)^{-1},$$

where the $\mathbb{X}(p)$ for primes p are the uniform, independent and identically distributed random variables on the unit circle in $\mathbb{C}$. The product converges almost surely for $\sigma > 1/2$ by Kolmogorov's three series theorem. Our main question is how well the distribution of $\zeta(\sigma, \mathbb{X})$ approximate that of the Riemann zeta function for $1/2 < \sigma < 1$.

Consider two measures

$$\Phi_{\zeta, T}(\sigma, \mathcal{B}) := \frac{1}{T} \operatorname{meas}\{t \in [T, 2T]: \log \zeta(\sigma + it) \in \mathcal{B}\}$$

and

$$\Phi_{\zeta}^{\operatorname{rand}}(\sigma, \mathcal{B}) := \mathbb{P}(\log \zeta(\sigma, \mathbb{X}) \in \mathcal{B})$$

for a Borel set $\mathcal{B}$ in $\mathbb{C}$. Define the discrepancy between the above two measures by

$$\mathbf{D}_{\zeta}(\sigma) := \sup_{\mathcal{R}} |\Phi_{\zeta, T}(\sigma, \mathcal{R}) - \Phi_{\zeta}^{\operatorname{rand}}(\sigma, \mathcal{R})|,$$

ON THE BEHAVIOR OF THE SOLUTION TO THE DEGASPERIS-PROCESI EQUATION WITH THE CORIOLIS EFFECT

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ABSTRACT. In this paper, we consider the Degasperis-Procesi equation with the Coriolis effect. We demonstrate a rigorous justification of the solution of the classical Degasperis-Procesi equation as an approximation to that of the Degasperis-Procesi equation with the Coriolis effect in a long time scale when the effect of the Earth's rotation vanishes. Also, nonexistence of the classical Camassa-Holm type peaked solitary waves is investigated.

1. Introduction. This paper is concerned with the following Degasperis-Procesi equation with the Coriolis effect:

$$u_t - \beta\mu u_{xxt} + cu_x + 4\alpha\epsilon uu_x - \beta_0\mu u_{xxx} + w_1\epsilon^2 u^2 u_x + w_2\epsilon^3 u^3 u_x \\ = \alpha\beta\epsilon\mu(3u_x u_{xx} + uu_{xxx}), \quad (1)$$

for the function $u(t, x)$ of time t and a single spatial variable x , where the parameter Ω is the constant rotational frequency due to the Coriolis effect, and two fundamental dimensionless parameters ϵ and μ are the amplitude parameter and the shallowness (long wavelength) parameter, respectively. The other constants appearing in the equation are defined by

$$c = \sqrt{1 + \Omega^2} - \Omega, \quad \alpha = \frac{3c^2}{4(1 + c^2)}, \quad \beta = \frac{4(3c^4 + 8c^2 - 1)}{27(c^2 + 1)^2}, \quad \beta_0 = \frac{c(3c^4 + 23c^2 - 4)}{27(c^2 + 1)^2}, \\ w_1 = \frac{-3c(c^2 - 1)(c^2 - 2)}{2(1 + c^2)^3}, \quad w_2 = \frac{(c^2 - 2)(c^2 - 1)^2(8c^2 - 1)}{2(1 + c^2)^5}.$$

This equation can be regarded as a rotation-Degasperis-Procesi (R-DP) equation analogous to the rotation-Camassa-Holm (R-CH) equation [14, 31], which was recently derived from the irrotational two-dimensional equatorial shallow-water equation in the presence of the Coriolis effect in a rotating frame. The Coriolis effect induced by the earth's rotation has an important role in understanding the dynamics of the atmosphere and of the ocean. It is noted that the Coriolis effect cannot be ignored when considering wave motions which appear on a wide range of spatial and temporal scales [5, 6, 11]. Several attempts have been made recently in modeling of equatorial shallow waters with the Coriolis effect in different nonlinear regimes

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Key words and phrases. Rotation-Degasperis-Procesi equation, Coriolis effect.

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Research Article

Byungsoo Moon* and Chao Yang

Peaked solitary waves and shock waves of the Degasperis-Procesi-Kadomtsev-Petviashvili equation

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Abstract: In this study, we establish the existence and nonexistence of smooth and peaked solitary wave solutions (or periodic) to the Degasperis-Procesi-Kadomtsev-Petviashvili (DP-KP) equation with a weak transverse effect. We have also shown that DP-KP equation possesses periodic shock waves similar to that of the Degasperis-Procesi equation.

Keywords: solitary waves, shock waves, Degasperis-Procesi-Kadomtsev-Petviashvili equation, Degasperis-Procesi equation

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1 Introduction

In this work, we show the existence of peaked solitary waves and shock waves of the Degasperis-Procesi-Kadomtsev-Petviashvili (DP-KP) equation

$$(u_t - u_{xxt} + \kappa u_x + 4uu_x - (3u_x u_{xx} + uu_{xxx}))_x + u_{yy} = 0, \quad (1.1)$$

where κ is a real parameter and the real function $u(t, x, y)$ depends on the spatial variables $x, y \in \mathbb{R}$ and the temporal variable $t > 0$. Equation (1.1) may be viewed as the Degasperis-Procesi (DP) counterpart of the two-dimensional generalization of the Korteweg-de Vries (KdV) equation [19] in much the same way as Camassa-Holm-Kadomtsev-Petviashvili (CH-KP) equation [15], which was formally derived from the incompressible and irrotational three-dimensional shallow water under the CH regime. Local well-posedness, formation of singularities, existence of traveling wave solutions, and the Liouville-type property for CH-KP equation were presented in [15]. The recent related results for other models with respect to transverse effects can be found in [3,13,16].

In the case of no y -dependence, equations (1.1) and CH-KP equation take the form of the integrable DP equation [9]

$$u_t - u_{xxt} + \kappa u_x + 4uu_x - (3u_x u_{xx} + uu_{xxx}) = 0 \quad (1.2)$$

and the Camassa-Holm (CH) shallow-water equation [2,12]

$$u_t - u_{xxt} + \kappa u_x + 3uu_x - (2u_x u_{xx} + uu_{xxx}) = 0, \quad (1.3)$$

respectively.

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Path description for q -characters of fundamental modules in type C

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ABSTRACT

In this paper, we investigate the behavior of monomials in the q -characters of the fundamental modules over a quantum affine algebra of untwisted type C. As a result, we give simple closed formulae for the q -characters of the fundamental modules in terms of sequences of vertices in $\mathbb{R}^2$, so-called paths, with an admissible condition. This may be viewed as a type C analog of the path description of q -characters in types A and B due to Mukhin–Young.

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1. Introduction

Let $\mathfrak{g}$ be a finite-dimensional simple Lie algebra over $\mathbb{C}$. We denote by I an index set of the simple roots for $\mathfrak{g}$. Let $\widehat{\mathfrak{g}}$ be the associated affine Lie algebra of untwisted type [20]. Let $U_q(\widehat{\mathfrak{g}})$ be the subquotient of the quantum affine algebra corresponding to $\widehat{\mathfrak{g}}$, that is, without the degree operator. The notion of q -characters for finite-dimensional $U_q(\widehat{\mathfrak{g}})$ -modules, which is defined in [11], is a refined analog of the ordinary character of finite-dimensional $\mathfrak{g}$ -modules. More precisely, the q -character homomorphism (simply called q -character) is an injective ring homomorphism from the Grothendieck ring of the category of finite-dimensional $U_q(\widehat{\mathfrak{g}})$ -modules into a ring of Laurent polynomials with infinitely many variables, denoted by $Y_{i,a}$ for $i \in I$ and $a \in \mathbb{C}^\times$.

The q -character is a useful tool for studying finite-dimensional $U_q(\widehat{\mathfrak{g}})$ -modules [10, 11, 16, 17]. Furthermore, it has been widely used and studied in several viewpoints, such as crystal bases [22, 31], cluster algebras [18], and integrable systems [11]. Despite its importance, it still seems to be a challenging problem to find explicit *closed* formulae for the q -characters of *arbitrary* finite-dimensional simple $U_q(\widehat{\mathfrak{g}})$ -modules, so there are several works which provide formulae *depending on $\mathfrak{g}$ and what kind of $U_q(\widehat{\mathfrak{g}})$ -modules*.

For example, for simply-laced types, it was known in [31, 32] that arbitrary simple modules could be essentially computed by an analog of the Kazhdan–Lusztig algorithm, where closed formulae for the q -characters of standard modules are given in terms of corresponding classical crystals. In [4, 5], Chari–Moura gave closed formulae for the q -characters of fundamental modules when $\mathfrak{g}$ is of classical type by using the invariance of a braid group action on ℓ -weights of fundamental modules. Note that the invariance is available only when $\mathfrak{g}$ is of classical type.

Since the q -character is defined as in (3.3), it is natural to associate a monomial with a sequence of vertices in $\mathbb{R}^2$. In [25], when $\mathfrak{g}$ is of type A and B, Mukhin and Young provided nice formulae of q -characters for a certain family of finite-dimensional $U_q(\widehat{\mathfrak{g}})$ -modules, so-called snake modules, in terms



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Research Paper

Crystal bases of parabolic Verma modules over the quantum orthosymplectic superalgebras [☆]

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ABSTRACT

We show that there exists a unique crystal base of a parabolic Verma module over a quantum orthosymplectic superalgebra, which is induced from a q -analogue of a polynomial representation of a general linear Lie superalgebra.

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1. Introduction

Let $\mathfrak{g}$ be a classical Lie superalgebra. It is a natural question whether there is a crystal base of a highest weight representation of a quantized enveloping algebra $U_q(\mathfrak{g})$

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Quantization of Virtual Grothendieck Rings and Their Structure Including Quantum Cluster Algebras

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Abstract: The quantum Grothendieck ring of a certain category of finite-dimensional modules over a quantum loop algebra associated with a complex finite-dimensional simple Lie algebra $\mathfrak{g}$ has a quantum cluster algebra structure of skew-symmetric type. Partly motivated by a search of a ring corresponding to a quantum cluster algebra of *skew-symmetrizable* type, the quantum *virtual* Grothendieck ring, denoted by $\mathfrak{K}_q(\mathfrak{g})$, is recently introduced by Kashiwara and Oh (Math Z 303(2):42, 2023) as a subring of the quantum torus based on the (q, t) -Cartan matrix specialized at $q = 1$. In this paper, we prove that $\mathfrak{K}_q(\mathfrak{g})$ indeed has a quantum cluster algebra structure of skew-symmetrizable type. This task essentially involves constructing distinguished bases of $\mathfrak{K}_q(\mathfrak{g})$ that will be used to make cluster variables and generalizing the quantum T -system associated with Kirillov–Reshetikhin modules to establish a quantum exchange relation of cluster variables. Furthermore, these distinguished bases naturally fit into the paradigm of Kazhdan–Lusztig theory and our study of these bases leads to some conjectures on quantum positivity and q -commutativity.

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Limit of Bergman kernels on a tower of coverings of compact Kähler manifolds

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Abstract

We prove the convergence of the Bergman kernels and the L^2 -Hodge numbers on a tower of Galois coverings $\{X_j\}$ of a compact Kähler manifold X converging to an infinite Galois (not necessarily universal) covering $\tilde{X}$. We also show that, as an application, sections of canonical line bundle K_{X_j} for sufficiently large j give rise to an immersion into some projective space, if so do sections of $K_{\tilde{X}}$.

1 Introduction

Let $\{X_j\}$ be a tower of Galois coverings of a complex manifold X converging to an infinite Galois covering $\tilde{X}$ of X . A tower of coverings is said to be *Bergman stable* if the pull-back of the Bergman kernels of X_j converges locally uniformly to that of $\tilde{X}$ as $j \rightarrow \infty$. A famous theorem by Kazhdan [9] states that a tower of coverings of compact Riemann surfaces with genus $g \geq 2$ converging to *the universal covering* is Bergman stable (see McMullen [13] and Rhodes [15] for other proofs). We remark that in contrast, Ohsawa [14] constructed an example of a tower of *non*-Galois coverings which is not Bergman stable.

Recently, Baik, Shokrieh and Wu generalized Kazhdan's theorem where $\tilde{X}$ is *any infinite Galois covering* of a compact Riemann surface X (see Theorem A in [1]). The purpose of this work is to prove the following generalization for higher dimensional complex manifolds.

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Products of generalized n -projections

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ABSTRACT

A Hilbert space operator $A \in \mathcal{B}(\mathcal{H})$ is a generalized n -projection, $A \in (G - n - P)$, if $A^{*n} = A$. The product AB of a commuting pair A, B of $(G - n - P)$ -operators is a $(G - n - P)$ -operator. The converse fails. We prove that if $\|AB\| = \|A\| \|B\|$ and $\sigma_a(AB) = \sigma_a(A)\sigma_a(B)$, then $AB \in (G - n - P)$ implies $\frac{A}{\|A\|}, \frac{B}{\|B\|}$ are $(G - n - P)$ if and only if A and B are normal operators. Translated to tensor products (and upon identifying the tensor product $A \otimes B$ with the left-right multiplication operator $\mathcal{E}_{A,B^*}$ acting on the Hilbert–Schmidt bimodule $\mathcal{C}_2(\mathcal{H})$) this says that $A \otimes B$ (resp., $\mathcal{E}_{A,B^*}$) is a $(G - n - P)$ -operator implies $\frac{A}{\|A\|}, \frac{B}{\|B\|}$ are $(G - n - P)$ if and only if A and B are normal operators.

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1. Introduction

Let $\mathcal{B}(\mathcal{H})$ denote the algebra of operators, i.e. bounded linear transformations, on an infinite dimensional complex Hilbert space $\mathcal{H}$ into itself. An operator $A \in \mathcal{B}(\mathcal{H})$, with adjoint A^* , is said to be a *generalized n -projection*, denoted $A \in (G - n - P)$, if $A^{*n} = A$. The concept of generalized 2-projections on a finite dimensional Hilbert space was introduced by Gross and Trenkler [1], who proved that a square matrix $A \in (G - 2 - P)$ if and only if (i) $A^4 = A$; (ii) A is normal; (iii) A is a partial isometry. Baksalary and Liu [2] improved upon this characterization proving that (i) along with either of the conditions (ii) and (iii) is sufficient to characterize the class of $(G - 2 - P)$ -operators in $\mathcal{B}(\mathcal{H})$. Du and Li [3] used spectral theory of Hilbert space operators to delve deeper into the geometry of $(G - n - P)$ -operators, and a generalization of the results of [3] is to be found in Lebtahi and Thome [4].

For operators $A, B \in \mathcal{B}(\mathcal{H})$, define the operators L_A and $R_B \in \mathcal{B}(\mathcal{B}(\mathcal{H}))$ of left multiplication by A and right multiplication by B by $L_A(X) = AX$ and $R_B(X) = XB$. Let $\mathcal{C}_2(\mathcal{H})$ denote the Hilbert–Schmidt class, equipped with its usual inner product $\langle A, B \rangle = \text{trace}(AB^*)$. If the elementary operator $\mathcal{E}_{A,B} = L_A R_B \in \mathcal{B}(\mathcal{C}_2(\mathcal{H}))$, $\mathcal{E}_{A,B}(T) = ATB$, then



Infinitely Many Small Energy Solutions to Nonlinear Kirchhoff–Schrödinger Equations with the p -Laplacian

In Hyoun Kim¹ · Yun-Ho Kim²

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Abstract

This paper is devoted to deriving the multiplicity result of solutions to the nonlinear elliptic equations of Kirchhoff–Schrödinger type on a class of a nonlocal Kirchhoff coefficient which slightly differs from the previous related works. More precisely, the main purpose of this paper, under the various conditions for a nonlinear term, is to show that our problem has a sequence of infinitely many small energy solutions. In order to obtain such a multiplicity result, the dual fountain theorem is used as the primary tool.

Keywords p -Laplacian · Kirchhoff function · Weak solutions · Dual fountain theorem

Mathematics Subject Classification 35D30 · 35J20 · 35J60 · 47J30

1 Introduction

The present paper is devoted to the multiplicity result of nontrivial weak solutions to the p -Laplacian equations of Kirchhoff–Schrödinger type:

$$-M\left(\int_{\mathbb{R}^N} |\nabla v|^p \, dx\right) \operatorname{div}(|\nabla v|^{p-2} \nabla v) + \mathfrak{P}(x) |v|^{p-2} v = \lambda h(x, v) \quad \text{in } \mathbb{R}^N, \quad (P_\lambda)$$

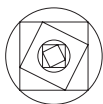
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TENSOR PRODUCTS OF ALUTHGE TRANSFORMS AND A -ADJOINTS OF m -ISOMETRIC OPERATORS

BHAGWATI P. DUGGAL AND IN HYOUN KIM

Given an m -isometric Hilbert space operator $A \in \mathcal{B}(\mathcal{H})$, $\Delta_{A^*, A}^m(I) = \sum_{j=0}^m (-1)^j \binom{m}{j} A^{*j} A^j = 0$, with polar decomposition $A = U|A|$, the Aluthge transform $\tilde{A} = |A|^{1/2} U |A|^{1/2}$ preserves almost all the spectral properties of A . However, the m -isometric property of an operator neither implies nor is implied by the m -isometric property of its Aluthge transform. The operator A has an $|A|$ -adjoint $\mathcal{A}$, $\mathcal{A}^* = [A]^* = U^* |A|$. If A_i , $i = 1, 2$, doubly commute and $\tilde{A}_i$ (resp. $\mathcal{A}_i$) is strict m_i -isometric, then $\widetilde{A_1 A_2}$ (resp. $\mathcal{A}_1 \mathcal{A}_2$) is strict $(m_1 + m_2 - 1)$ -isometric. The converse fails for products $A_1 A_2$, $\tilde{A}_1 \tilde{A}_2$ and $\mathcal{A}_1 \mathcal{A}_2$, but has a positive answer for tensor products $A_1 \otimes A_2$, $\tilde{A}_1 \otimes \tilde{A}_2$, $\mathcal{A}_1 \otimes \mathcal{A}_2$ (and their Hilbert–Schmidt class identifications with the elementary operators $L_{A_1} R_{A_2^*}$, $L_{\tilde{A}_1} R_{\tilde{A}_2^*}$ and $L_{\mathcal{A}_1} R_{\mathcal{A}_2^*}$); if $S \otimes T$, where $S \otimes T$ stands for either of the three tensor products above, is strict m -isometric, then there exist scalars c and d , $|cd| = 1$, and positive integers m_1 and m_2 , $m = m_1 + m_2 - 1$, such that cS is strict m_1 -isometric and dT is strict m_2 -isometric.

1. Introduction

Let $\mathcal{B}(\mathcal{H})$ denote the algebra of bounded linear transformations on an infinite-dimensional complex Hilbert space $\mathcal{H}$ that map into $\mathcal{H}$. A generalisation of isometric operators $A \in \mathcal{B}(\mathcal{H})$ is obtained by calling A m -isometric, denoted by $A \in m\text{-isometric}$, if

$$\Delta_{A^*, A}^m(I) = (I - L_{A^*} R_A)^m(I) = \sum_{j=0}^m (-1)^j \binom{m}{j} A^{*j} A^j = 0,$$

where $L_{A^*}, R_A \in B(\mathcal{B}(\mathcal{H}))$ are, respectively, the operator $\tilde{L}_{A^*}(X) = A^*X$ of left multiplication by A^* and the operator $R_A(X) = XA$ of right multiplication by A . Motivated by the work of W. Helton, the concept of m -isometric operators was introduced by J. Agler [1], and a study of the structure of the class of m -isometric operators was initiated by Agler and Stankus in [2]. This class of operators has since been studied extensively. If an $A \in \mathcal{B}(\mathcal{H})$ has the polar decomposition $A = U|A|$, then the Aluthge transform $\tilde{A}$ of A is the operator $\tilde{A} = |A|^{1/2} U |A|^{1/2} \in \mathcal{B}(\mathcal{H})$ [10]. The Aluthge transforms preserve, often improve upon, many a spectral property of the operator. However, as Botelho and Jamison [6] point out in their study of elementary operators and Aluthge transforms, the m -isometric property of an operator neither implies nor is implied by the m -isometric property of its Aluthge transform.

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Keywords and phrases: left/right multiplication operator, m -isometric operator, Aluthge transform, P -adjoint, tensor product, Hilbert–Schmidt operator.

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m -symmetric Operators with Elementary Operator Entries

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Abstract. Given Banach space operators A, B , let $\delta_{A,B}$ denote the generalised derivation $\delta(X) = (L_A - R_B)(X) = AX - XB$ and $\Delta_{A,B}$ the length two elementary operator $\Delta_{A,B}(X) = (I - L_A R_B)(X) = X - AXB$. This note considers the structure of m -symmetric operators $\delta_{\Delta_{A_1, B_1}, \Delta_{A_2, B_2}}^m(I) = (L_{\Delta_{A_1, B_1}} - R_{\Delta_{A_2, B_2}})^m(I) = 0$. It is seen that there exist scalars $\lambda_i \in \sigma_a(B_i)$, $1 \leq i \leq 2$, such that $\delta_{\lambda_1 A_1, \lambda_2 A_2}^m(I) = 0$. Translated to Hilbert space operators A and B this implies that if $\delta_{\Delta_{A^*, B^*}, \Delta_{A, B}}^m(I) = 0$, then there exists $\bar{\lambda} \in \sigma_a(B^*)$ such that $\delta_{(\bar{\lambda} A)^*, \lambda A}^m(I) = 0 = \delta_{\bar{\lambda} B, \lambda B^*}^m(I)$. We prove that the operator $\delta_{\Delta_{A^*, B^*}, \Delta_{A, B}}^m$ is compact if and only if (i) there exists a real number α and finite sequences (i) $\{a_j\}_{j=1}^n \subseteq \sigma(A)$, $\{b_j\}_{j=1}^n \subseteq \sigma(B)$ such that $a_j b_j = 1 - \alpha$, $1 \leq j \leq n$; (ii) decompositions $\oplus_{j=1}^n \mathcal{H}_j$ and $\oplus_{j=1}^n \mathbb{H}_j$ of $\mathcal{H}$ such that $\oplus_{j=1}^n (A - a_j I)|_{\mathcal{H}_j}$ and $\oplus_{j=1}^n (B - b_j I)|_{\mathbb{H}_j}$ are nilpotent. If $\delta_{\Delta_{A^*, B^*}, \Delta_{A, B}}^m(I) = 0$ implies $\delta_{\Delta_{A^*, B^*}, \Delta_{A, B}}(I) = 0$, then A and B satisfy a (Putnam-Fuglede type) commutativity theorem; conversely, a sufficient condition for $\delta_{\Delta_{A^*, B^*}, \Delta_{A, B}}^m(I) = 0$ to imply $\delta_{\Delta_{A^*, B^*}, \Delta_{A, B}}(I) = 0$ is that λA and $\bar{\lambda} B$ satisfy the commutativity property for scalars $\lambda \in \sigma_a(B^*)$. An analogous result is seen to hold for the operators $\Delta_{\delta_{A^*, B^*}, \delta_{A, B}}^m$ and $\Delta_{\delta_{A^*, B^*}, \delta_{A, B}}(I)$. Perturbation by commuting nilpotents is considered.

Mathematical subject classification . Primary 47B47, 47A80; Secondary 47A80, 47B10.

Keywords. Banach space, left/right multiplication operator, generalized derivation, elementary (length two) operator, m symmetric operator, compact operator, commutativity property, perturbation by an operator, tensor product.



Existence Results and L^∞ -Bound of Solutions to Kirchhoff–Schrödinger–Hardy Type Equations Involving Double Phase Operators

Jun-Hyuk Ahn, In Hyoun Kim, Yun-Ho Kim , and Shengda Zeng

Abstract. This paper is concerned with several multiplicity results of non-trivial weak solutions to the Kirchhoff–Schrödinger type double phase problems with Hardy potentials. The main features of the paper are the presence of non-local Kirchhoff coefficients and the Hardy potential, the lack of compactness of the Euler–Lagrange functional, and the uniform boundedness for weak solutions. To establish such multiplicity results, we employ the fountain theorem and the dual fountain theorem as the primary tools, respectively. Finally, we demonstrate the L^∞ -bound for any possible weak solution by taking into consideration the De Giorgi iteration method and a truncated energy technique.

Mathematics Subject Classification. 35B38, 35D30, 35J10, 35J20, 35J62.

Keywords. Double phase problems, Musielak–Orlicz–Sobolev spaces, variational methods, multiple solutions, De Giorgi iteration.

1. Introduction

The double-phase operator, which is the natural generalization of the p -Laplace operator, has been extensively investigated by many researchers. The research interest in the differential equations and variational problems with the double-phase operator has gained prominence due to their relevance in diverse fields of mathematical physics, such as strongly anisotropic materials, Lavrentiev’s phenomenon, plasma physics, biophysics, and chemical reactions, etc.; see [57, 58]. With respect to the theory of regularity for double phase functionals,

Research Article

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Nilpotent perturbations of m -isometric and m -symmetric tensor products of commuting d -tuples of operators

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Abstract: If $\mathbb{T}_1$ and $\mathbb{T}_2$ are commuting d -tuples of Hilbert space operators in $B(\mathcal{H})^d$ such that $(\mathbb{T}_1^* \otimes I + I \otimes \mathbb{T}_2^*, \mathbb{T}_1 \otimes I + I \otimes \mathbb{T}_2)$ is strictly m -isometric (resp., m -symmetric) for some positive integer m , then there exist a scalar d -tuple λ and positive integers m_i , $1 \leq i \leq 2$, such that $m = m_1 + 2m_2 - 2$, $(\mathbb{T}_1^* + \bar{\lambda}, \mathbb{T}_1 + \lambda)$ is m_1 -isometric, and $\mathbb{T}_2 - \lambda$ is m_2 -nilpotent (resp., $m = m_1 + m_2 - 1$, $(\mathbb{T}_1^* + \bar{\lambda}, \mathbb{T}_1 + \lambda)$ is m_1 -symmetric and $(\mathbb{T}_2^* - \bar{\lambda}, \mathbb{T}_2 - \lambda)$ is m_2 -symmetric).

Keywords: Banach space, left/right multiplication operator, m -isometric and m -symmetric commuting d -tuples of operators, tensor product of operators

MSC 2020: 47A05, 47A55, 47A11, 47B47

1 Introduction

Let $B(\mathcal{X})^d$ (resp., $B(\mathcal{H})^d$) denote the product of d -copies of the algebra of operators, i.e., bounded linear transformations, on an infinite dimensional complex Banach space $\mathcal{X}$ into itself (resp., on an infinite dimensional complex Hilbert space $\mathcal{H}$ into itself). A d -tuple $\mathbb{A} = (A_1, \dots, A_d) \in B(\mathcal{X})^d$ is a commuting d -tuple if $[A_i, A_j] = A_i A_j - A_j A_i = 0$ for all $1 \leq i, j \leq d$. Recall from Gleason and Richter [1] that a commuting d -tuple $\mathbb{A} \in B(\mathcal{H})^d$ is m -isometric if

$$\Delta_{\mathbb{A}, \mathbb{A}}^m(I) = \sum_{j=0}^m (-1)^j \binom{m}{j} \sum_{|\beta|=j} \frac{j!}{\beta!} \mathbb{A}^{*\beta} \mathbb{A}^\beta = 0,$$

where $\beta = (\beta_1, \dots, \beta_d)$, β_i are non-negative integers for all $1 \leq i \leq d$, $|\beta| = \sum_{i=1}^d \beta_i$, $\beta! = \prod_{i=1}^d \beta_i!$, $\mathbb{A}^\beta = \prod_{i=1}^d A_i^{\beta_i}$, and $\mathbb{A}^{*\beta} = \prod_{i=1}^d A_i^{*\beta_i}$. Generalizing this to commuting d -tuple pairs $(\mathbb{A}, \mathbb{B})$ of Banach space operators, the pair $(\mathbb{A}, \mathbb{B})$ is said to be m -isometric if

$$\Delta_{\mathbb{A}, \mathbb{B}}^m(I) = \sum_{j=0}^m (-1)^j \binom{m}{j} \left(\sum_{|\beta|=j} \frac{j!}{\beta!} \mathbb{A}^\beta \mathbb{B}^\beta \right) = 0$$

[1–3]. Analogously [3], the commuting d -tuple $(\mathbb{A}, \mathbb{B})$ is said to be m -symmetric if

$$\delta_{\mathbb{A}, \mathbb{B}}^m(I) = \sum_{j=0}^m (-1)^j \binom{m}{j} \left(\sum_{i=1}^d A_i \right)^{m-j} \left(\sum_{i=1}^d B_i \right)^j = 0.$$

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ON THE ADAPTED PARTIAL DIFFERENTIAL EQUATION FOR GENERAL DIPLOID MODEL OF SELECTION AT A SINGLE LOCUS

WON CHOI

ABSTRACT. Assume that at a certain locus there are three genotypes and that for every one progeny produced by an $I^A I^A$ homozygote, the heterozygote $I^A I^B$ produces. W. Choi found the adapted partial differential equations for the density and operator of the frequency for one gene and applied this adapted partial differential equations to several diploid model. Also, he found adapted partial differential equations for the diploid model against recessive homozygotes and in case that the allele frequency occurs after one generation of selection when there is no dominance. (see. [1, 2]).

In this paper, we find the adapted partial equations for the model of selection against heterozygotes and in case that the allele frequency changes after one generation of selection when there is overdominance. Finally, we shall find the partial differential equation of general type of selection at diploid model and it also shall apply to actual examples. This is a very meaningful result in that it can be applied **in any model**.

1. Introduction

Assume that at a certain locus there are three genotypes and that for every one progeny produced by an $I^A I^A$ homozygote, the heterozygote $I^A I^B$ produces. To calculate a genotypic frequency, we add up the number of individuals possessing a genotype and divide by the total number of individuals in the sample N . The gene pool of a population can be represented in terms of allelic frequencies. Allelic frequencies can be calculated from the numbers or the frequencies of the genotypes. To calculate the allelic frequency from the numbers of genotypes, we count the number of copies of a particular allele present among the genotypes and divide by the total number of all alleles in the sample. ([5])

Suppose that N_{AA} , N_{AB} and N_{BB} are the numbers of $I^A I^A$, $I^A I^B$ and $I^B I^B$ respectively and N is the total number of populations. For a locus with only two alleles, the frequencies of the alleles are usually represented by p and q , respectively. To calculate the number of copies of the allele in the numerator of the equation, we add twice the number of homozygotes ($2N_{AA}$ or $2N_{BB}$) to the number of heterozygotes

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Long-range ordered graphitic structure in silk fibers delaminated using dopamine and thermal treatment for super-flexible electronic textiles: Possible applications for magnetic and thermoelectric textiles

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Abstract

Exfoliation has been used to obtain 2D materials with attractive properties, like graphene. The layered structure of silk and its structural transition to a pseudo-graphitic structure by heat treatment has attracted scientific interest because of its potential applications. Here, we report that intercalating the silk layers with polydopamine (PDA) followed by thermal treatment delaminates the silk fibers. Cylindrical silk fibers with a diameter of 10 μm changed to graphitic ribbon-like structures with widths of approximately 65 nm. This structural transition was systematically investigated using thermally treated PDA-embedded silks at temperatures from 800 to 1450 °C. The graphitic structures start to be observed at 1000 °C, a much lower heat treatment temperature than previous studies using pure silk. Long-range ordered graphitic structures with a lattice distance of 3.4 Å formed most clearly at 1300 °C. Ab initio molecular dynamics calculations confirmed the microscopic origin of the experimental results. This method allows the fabrication of super-flexible, comparable to pure silk, and high-electrically conductive (277.3 S·cm⁻¹) e-textiles. These thermally treated PDA-embedded silks were also functionalized with Fe₂O₃ and Bi₂Te₃ powders using the attachment ability of PDA. This work provides evidence that PDA plays a role in delaminating silk fibers and opens the potential for various applications.

Keywords Commercial silk · Polydopamine · Delamination of silk · Super-flexible electronic textiles

1 Introduction

After interesting properties were found in 2D materials such as graphene and transition metal dichalcogenides, growing attention has been focused on the delamination of layered

materials to achieve low dimensionality [1–11]. The delamination has been achieved by various methods, for example, mechanical, electrochemical, liquid-phase, and green chemistry approaches [1, 2, 12–15]. In addition to delamination to obtain 2D materials, the intentional thinning of materials can provide advantages of stretchability and flexibility [16, 17]

Hyun-Seok Jang, Shinik Kim, and Ikpyeong Park contributed equally to the work.

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Micro-scale gas tungsten arc welding on invar sheet

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Abstract

Manipulation of metal microstructures via welding technique has attracted many interests due to its various applications for microscopic electronic circuits and photo-electronic structures. Refilling deletion of structures is challenging in sculpting metal microstructure. We propose the micro-size gas tungsten arc welding (GTAW) technique to overcome these challenges. The welded area of $\sim 10 \text{ s } \mu\text{m}$ are achieved by using an etched $100 \text{ } \mu\text{m}$ W wire with optimized arc current and voltage control. The spatial size and depth of the welded spot in the invar surface show dependency on the arc current and voltages under proper Ar gas flow. We believe that these results can provide technical intuition for developing many industrial applications.

Keywords Gas tungsten arc welding · White-light interferometry · Metal structure · Invar welding · Precision measurement

1 Introduction

As electronic structures have shrunk to microscopic levels, many techniques to create smaller welding spots have attracted interest in various industrial fields [1–3]. Micro-structures such as on-chip wiring [4–6], micro-structured metal mask [7–9], photonic meta-structure [10] can be achieved through laser machining [11], wet etching [12, 13], and photo-chemical methods [14–16]. While these techniques offer significant flexibility in eliminating materials to create designed shapes, adding material remains challenging. In this letter, we propose a gas tungsten arc welding (GTAW) method to remove and add material to modify the metal micro-structures.

Invar 42, an alloy composed of 42% nickel and 58% iron, is renowned for its extremely low coefficient of thermal

expansion. Therefore, it was selected to minimize the thermal expansion artifacts during the arc welding process [17–20]. This unique property makes invar highly valuable in the manufacture of precision instruments, measuring devices, and standard equipment [21–24].

Laser welding is a typical method to drill thin invar sheets due to its controllability, resolution, and cleanliness [25, 26]. However, laser welding in metal micro-structure fabrication generally involves laser ablation. Therefore, over-drilling can occur during the drilling process to fabricate the metal micro-structures, leading to structural defects. Additionally, while it is possible to re-drill clogged holes during the cleaning process of the contaminated metal micro-structure, refilling missing lines is challenging, often resulting in the structures being discarded. This difficulty lowers manufacturing yield in industrial applications. Attempts to fill using laser filler welding can result in ablated fumes that can be redeposited on the metal surface as defects [27]. Filling material via welding requires an additional filler wire close to the surface with high precision, and the laser's incidence angle restricts the filler wire's feeding angle. As a result, the laser beam can be deflected, so laser welding cannot be the universal solution to fix metal structures [28].

We developed the micro-size GTAW with an arc state between invar sheet and tungsten wire to overcome these limitations. The weld region is around $10 \text{ s } \mu\text{m}$, which is ten times smaller than conventional micro-GTAW methods [29]. The voltage drop during the arc state is monitored by an oscilloscope and controlled by increasing the circuit's

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Photoluminescence of MoS₂ on Plasmonic Gold Nanoparticles Depending on the Aggregate Size

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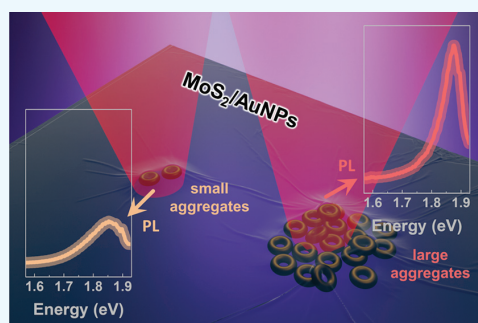


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Supporting Information

ABSTRACT: Transition metal dichalcogenides (TMDs) are promising candidates for ultrathin functional semiconductor devices. In particular, incorporating plasmonic nanoparticles into TMD-based devices enhances the light–matter interaction for increased absorption efficiency and enables control of device performance such as electronic, electrical, and optical properties. In this heterohybrid structure, manipulating the number of TMD layers and the aggregate size of plasmonic nanoparticles is a straightforward approach to tailoring device performance. In this study, we use photoluminescence (PL) spectroscopy, which is a commonly employed technique for monitoring device performance, to analyze the changes in electronic and optical properties depending on the number of MoS₂ layers and the size of the gold nanoparticle (AuNP) aggregate under nonresonant and resonant excitation conditions. The PL intensity in monolayer MoS₂/AuNPs increases as the size of aggregates increases irrespective of the excitation conditions. The strain induced by AuNPs causes a red shift, but as the aggregates grow larger, the effect of p-doping increases and the blue shift becomes prominent. In multilayer MoS₂/AuNPs, quenched PL intensity is observed under nonresonant excitation, while enhancement is noted under resonant excitation, which is mainly contributed by p-doping and LSPR, respectively. Remarkably, the alteration in the spectral shape due to resonant excitation is evident solely in small aggregates of AuNPs across all layers.



INTRODUCTION

Two-dimensional (2D) semiconducting materials have unique quantum physical properties, such as large exciton binding energy and a direct electronic band gap, owing to their atomically thin molecular structure. Hence, these materials allow us to design functional quantum electronic and photonic devices with ultrathin thickness. Specifically, 2D transition metal dichalcogenides (TMDs) exhibit extraordinary electronic, optical, and mechanical properties, making them primarily employed in optoelectronic and photonic device applications.^{1–3}

However, 2D TMDs have relatively low absorption efficiency due to their thin thickness, which limits the generation of photoexcited carriers. Since the resulting low photocurrent and photoluminescence (PL) restrict the overall performance of the device, the practical application of TMDs is inefficient. As a promising strategy for reducing absorption loss, combining TMDs with nanophotonic devices, such as meta-^{4–9} and plasmonic^{10–17} structures, has garnered significant attention. The heterohybrid structures have the potential to offer new functionalities and exploit synergistic effects through material interactions, which can highly improve the device performance and control the optical, electronic, and electrical properties.

2D TMDs integrated with plasmonic nanoparticles are one of the most beneficial heterohybrid structures.^{14–17} The plasmonic nanoparticles contribute to the control of device performance by enhancing the absorption efficiency through plasmonic light–matter interactions and providing additional charge channels and local strain. By placing nanoparticles underneath the 2D TMDs, it becomes feasible to induce the strain. The magnitude and direction of the strain applied to them can be regulated by exercising control over the size, shape, and arrangement of the nanoparticles. The induced strain to 2D TMDs leads to alternations in the crystal lattice with modified interatomic interactions, which give rise to the changed electronic and optical properties.^{18–21} Furthermore, plasmonic nanoparticles can also change the electrical properties of adjacent materials at contact interfaces. The band alignment that arises during contact introduces changes in the charge transport behavior and the redistribution of the

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Probing Inherent Optical Anisotropy in Substrates via Direct Nanoimaging of Mie Scattering

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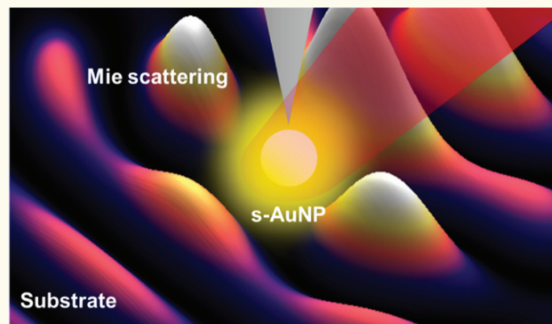
Article Recommendations



Supporting Information

ABSTRACT: In this study, we investigated the optical properties of a transition metal dichalcogenide (TMD) substrate via Mie-scattering-induced surface analysis (MISA). Employing near-field optical microscopy and finite-difference time-domain (FDTD) simulations, we systemically prove and directly visualize the Mie scattering of superspherical gold nanoparticles (s-AuNPs) at the nanoscale. Molybdenum disulfide substrates exhibited optical isotropy, while rhenium disulfide (ReS₂) substrates showed anisotropic behavior attributed to the interaction with incident light's electric field. Our study revealed substantial anisotropic trends in Mie scattering, particularly in the near-infrared energy range, with ReS₂ exhibiting more pronounced spectral and angular responses in satellite peaks. Our results emphasize the application of Mie scattering, exploring the optical properties of substrates and contributing to a deeper understanding of nanoscale light–matter interactions.

KEYWORDS: superspherical-AuNP, scattering-type scanning near-field optical microscope, Mie scattering, anisotropy, substrate



INTRODUCTION

The exploration of light–matter interactions at the nanoscale is pivotal in nanophotonics and materials science. Among the various light–matter interactions, Mie scattering describes the scattering of electromagnetic radiation by spherical particles and provides invaluable insights into subwavelength structures on a diffraction-limited scale. As Mie theory has been extensively used to explain and predict the optical properties of various materials, including nanoparticles,^{1–3} nanostructures,^{4–6} and metamaterials,^{4,5,7,8} researchers have utilized Mie theory to promote the development of cutting-edge technologies that bridge the classical and quantum optics of light–matter interactions.⁹ Because Mie scattering depends strongly on the size or shape of the particles, in-depth experimental studies with a high spatial resolution should be performed on single particles, along with theoretical simulations, to design or utilize the optical properties of materials.^{10–12} One way to achieve direct nanoimaging technology is scattering-type scanning near-field optical microscopy (s-SNOM).^{13–15} The s-SNOM has emerged as a powerful tool for probing the optical properties of nanoscale

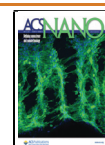
materials with unmatched spatial resolution because it is based on atomic force microscopy (AFM). This capability has contributed to the exploration of plasmonic single nanoparticles,^{16,17} polaritons,^{18–20} topological photonics²¹ and various nanoscale optical phenomena, offering a deeper understanding of the behavior of light–matter interactions.²² While theoretical simulations and spectroscopic techniques have been employed extensively to study Mie scattering,^{23–27} there is a notable limitation in the literature, mostly owing to the spatial resolution necessary for the direct experimental observation of Mie scattering phenomena. This discrepancy in scale has sparked a pressing need to bridge the gap between theory and experimentation, paving the way for more accurate and insightful studies of Mie scattering.

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High-speed lateral scanning white-light phase shift interferometry

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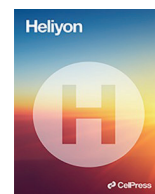
Abstract: In this study, we present lateral scanning white light interferometry (LS-WLI), where phase-shifting algorithms are applied to inspect the topography of a large field of view (FOV) with high-speed measurements. At a point, the interference signal must be acquired with a specific condition to adapt the phase-shifting algorithm. This means that all points have two points, of which the phase difference is $\pi/2$, when the number of points acquired in a phase period is multiple of 4, despite increasing the data points in a period. Consequently, stretching the fringe spacing in LS-WLI facilitates the application of phase-shift techniques, thereby enhancing stage speed, even with a fixed camera speed. Using the proposed method, we can successfully obtain a laterally expended topographic image as $5.25 \text{ mm} \times 1.25 \text{ mm}$, where the step height of the microstructure is 140 nm.

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1. Introduction

White light interferometry (WLI) has attracted industrial interests due to the ability to measure surface topography with various advantages such as high-precision vertical resolution, wide field of view (FOV), non-contact methods [1–10]. Unlike laser interferometry, which utilizes a coherent light source, WLI employs an incoherent light source with a short coherence length to resolve the height ambiguity for large-step-height region over 2π phase. These characteristics have led to efforts in the fabrication and manufacturing process of semiconductor-based devices to integrate WLI with other precision measurement equipment, such as atomic force microscopy [11]. Despite the efforts, WLI has limitations in monitoring the fabrication process at wafer scale due to a limited FOV. Therefore, image stitching methods have emerged as one of solution due to ability to obtain large FOV [12,13]. However, the image stitching methods involve some critical problems such as pixel mismatch and patternless intersection areas [14].

To overcome the problem of image stitching, lateral scanning white-light interferometry (LS-WLI) was proposed to measure topography with a wide FOV by moving the sample laterally without stitching images. Traditional LS-WLI has measured the interference signal with the off-axis motion of the laterally moving linear stage which causes intensity irregularities [15–17]. Therefore, the calibration of the stage motion is essential for obtaining accurate topography. T. Guo et al. developed a LS-WLI based on nanomeasuring machine for compensation of the error caused by off-axis motion [18]. However, the speed of the linear stage must be restrictively chosen within the limitations of the speed of camera [19,20]. Despite the technical importance of high-speed WLI, there has been limited research on LS-WLI with an increased speed while fixing a camera speed.



Research article

High-response room-temperature NO₂ gas sensor fabricated with thermally reduced graphene oxide-coated commercial cotton fabric

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Keywords:

NO₂ gas sensing

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ABSTRACT

Electronic textile-based gas sensors with a high response for NO₂ gas were fabricated using reduced graphene oxide (rGO)-coated commercial cotton fabric (rGOC). Graphene oxide (GO) was coated on cotton fabric by simply dipping the cotton into a GO solution. To investigate the relationship between the degree of reduction and the sensing response, the GO-coated fabrics were thermally reduced at various temperatures (190, 200, 300, and 400 °C). The change in the amount of oxygen functional groups on the rGOCs was observed by x-ray photoelectron spectroscopy, Raman spectroscopy, and x-ray diffraction patterns. The maximum sensing response of 45.90 % at 10 ppm of NO₂ gas at room temperature was exhibited by the rGOC treated at 190 °C, which was the lowest heat-treatment temperature. The high response comes from the greater amount of oxygen functional groups compared to other rGOC samples, and the tubular structure of the cotton.

1. Introduction

Interest in the real-time monitoring of harmful molecules has increased due to continuing efforts to monitor and address environmental pollution, and electronic textile (e-textile)-based gas sensors have attracted much attention for this application [1–7]. E-textiles not only have the inherent characteristics of textiles, being light weight, portable, flexible, with adaptable shapes but can also be electrically conducting. As a result, e-textiles are considered suitable for flexible, resistive gas sensors.

Among such applications, e-textile-based wearable NO₂ gas sensors using carbon nanotubes (CNTs) and graphene have been reported [8]. However, graphene and CNTs are presently not suitable for practical application, because of mass production difficulties, and relative cost-inefficiency [9]. In contrast, graphene oxide (GO) can be mass produced by Hummers' method. And because GO contains numerous oxygen functional groups (OFGs) including hydroxyl, epoxy groups, and carboxyl groups [10], it can also be easily attached to commercial textiles. GO also has a notable advantage for detecting NO₂ gas: the hydroxyl groups in GO are rotated to NO₂,

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RESEARCH ARTICLE

Self-supported VO₂ on polydopamine-derived pyroprotein-based fibers for ultrastable and flexible aqueous zinc-ion batteries

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Abstract

A conventional electrode composite for rechargeable zinc-ion batteries (ZIBs) includes a binder for strong adhesion between the electrode material and the current collector. However, the introduction of a binder leads to electrochemical inactivity and low electrical conductivity, resulting in the decay of the capacity and a low rate capability. We present a binder- and conducting agent-free VO₂ composite electrode using in situ polymerization of dopamine on a flexible current collector of pyroprotein-based fibers. The as-fabricated composite electrode was used as a substrate for the direct growth of VO₂ as a self-supported form on polydopamine-derived pyroprotein-based fibers (pp-fibers@VO₂(B)). It has a high conductivity and flexible nature as a current collector and moderate binding without conventional binders and conducting agents for the VO₂(B) cathode. In addition, their electrochemical mechanism was elucidated. Their energy storage is induced by Zn²⁺/H⁺ coininsertion during discharging, which can be confirmed by the lattice expansion, the formation of by-products including Zn_x(OTf)_y(OH)_{2x-y}·nH₂O, and the reduction of V⁴⁺ to V³⁺. Furthermore, the assembled

Jeong Seok Yeon, Sul Ki Park, and Shinik Kim contributed equally to this work.

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Article

Flexible Mechanical Sensors Fabricated with Graphene Oxide-Coated Commercial Silk

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Abstract: Many studies on flexible strain and pressure sensors have been reported due to growing interest in wearable devices for healthcare purposes. Here, we present flexible pressure and strain (motion) sensors prepared with only graphene oxide (GO) and commercial silk fabrics and yarns. The pressure sensors were fabricated by simply dipping the silk fabric into GO solution followed by applying a thermal treatment at 400 °C to obtain reduced GO (rGO). The pressure sensors were made from rGO-coated fabrics, which were stacked in three, five, and seven layers. A super-sensitivity of $2.58 \times 10^3 \text{ kPa}^{-1}$ at low pressure was observed in the seven-layer pressure sensor. The strain sensors were obtained from rGO-coated twisted silk yarns whose gauge factor was 0.307. Although this value is small or comparable to the values for other sensors, it is appropriate for motion sensing. The results of this study show a cost-effective and simple method for the fabrication of pressure and motion sensors with commercial silk and GO.

Keywords: commercial silk; graphene oxide; thermal treatment; pressure sensor; motion sensor



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1. Introduction

Flexible electronic components are steadily being developed for various applications, including electrically conductive textiles [1–4], thermoelectric textiles [5–7], solar cells [8], secondary batteries [9], and gas sensors [10–12]. Among them, flexible mechanical sensors (strain and pressure sensors) have been of interest because of their utility for sensing human body functions for healthcare purposes and for use in applications related to the Internet of Things (IoT). In particular, textile-based mechanical sensors have the advantage of being lightweight and low-cost and having superior flexibility [13–16]. Commercial textiles such as cotton, nylon, polyester, and silk have been used as flexible substrates for such sensors. However, other components are used to ensure high sensitivity and flexibility.

Cotton fabric has been decorated with Ag nanowires, Ag flowers, and Ag thread [17–20]; coatings of electrochemically exfoliated graphene film have been applied on cotton fabric [21]; and coatings of Ag have been applied to cotton yarn using Ag paste followed by sintering [22] to provide electrical conductivity. Likewise, electronic textiles for sensors fabricated with nylon and polyester have been created using conductive polymers [23], Au and MoS₂ coating [24], graphite-polyurethane (PU) coating applied to crochet knitted elastic [25], the screen printing of elastic conductive carbon ink on polyester fabric [26], the wet spinning of CNT/thermoplastic PU on silver-plated nylon electrodes [27], the coating of CNT/carbon black PU on fabric with Ag-coated conductive nylon fiber [28], and the spraying of multiwall CNT/TiO₂ conductive suspensions on nylon textiles [29]. Mxene has also been applied to textiles to improve sensitivity [30–34]. In the previous studies mentioned above, complex processes are inevitably required for the fabrication of the sensors.

The Impact of Supersaturated Electrode on Heterogeneous Lithium Nucleation and Growth Dynamics

Jimin Park, Ji In Jung, Son Ha, Do Hyun Kim, Hyun-Seok Jang, Byung Hoon Kim, Hyung-Kyu Lim, Hyoung-Joon Jin,* and Young Soo Yun*

Abstract: The concept of a lithiophilic electrode proves inadequate in describing carbon-based electrode materials due to their substantial mismatch in surface energy with lithium metal. However, their notable capacity for lithium chemisorption can increase active lithium concentration required for nucleation and growth, thereby enhancing the electrochemical performance of lithium metal anodes (LMAs). In this study, we elucidate the effects of the supersaturated electrode which has high active lithium capacity around equilibrium lithium potential on LMAs through an in-depth electrochemical comparison using two distinct carbon electrode platforms with differing carbon structures but similar two-dimensional morphologies. In the supersaturated electrode, both the dynamics and thermodynamic states involved in lithium nucleation and growth mechanisms are significantly improved, particularly under continuous current supply conditions. Furthermore, the chemical structures of the solid-electrolyte-interface layers (SEIs) are greatly influenced by the elevated surface lithium concentration environment, resulting in the formation of more conductive lithium-rich SEI layers. The improved dynamics and thermodynamics of surface lithium, coupled with the formation of enhanced SEI layers, contribute to higher power capabilities, enhanced Coulombic efficiencies, and improved cycling performances of LMAs. These results provide new insight into understanding the enhancements in heterogeneous lithium nucleation and growth kinetics on the supersaturated electrode.

Introduction

Amid the booming e-mobility sector, global initiatives are intensifying to enhance the energy densities of rechargeable lithium-ion batteries (LIBs).^[1–3] A pivotal challenge is the exploration of novel chemistries to transcend the capacity confines of traditional intercalation-based active electrode materials, which rely on storing charges within their crystalline structures.^[4,5] The host-free lithium metal anode (LMA) emerges as a leading contender for next-generation LIBs, acclaimed for its specific and volumetric capacities ($\sim 3,860 \text{ mAh g}^{-1}$ and $\sim 2,060 \text{ mAh cm}^{-3}$, respectively) alongside a low redox potential (-3.04 V vs. SHE).^[6,7] However, the LMA faces considerable challenges, such as inadequate Coulombic efficiency (CE), continuous electrolyte depletion, substantial volume expansion, severe surface deterioration, and safety risks.^[8–10] These issues primarily arise from uneven lithium deposition and dendritic metal growth. In response to these challenges, a variety of research strategies have been pursued, with the introduction of lithiophilic electrodes emerging as a particularly notable solution.^[11–13]

Lithiophilic electrodes are understood as a concept that encompasses electrodes with good lithium wettability from a macroscopic perspective or electrodes that contain a large number of atomic lithium adsorption sites from a microscopic viewpoint.^[14,15] Lithium alloy-forming materials, transition metal oxides, and metallic compounds are recognized for their superior lithium wettability.^[16–19] Extensive research has emphasized their effectiveness in promoting uniform and reversible electrochemical cycles of lithium deposition and dissolution.^[14–20] These positive effects are in concordance with classical nucleation theory (CNT), which emphasizes the significance of lower interfacial energy between the

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Raman-tensor analysis of phonon modes in (Pb, Bi)Sr₂CaCu₂O_{8+δ}

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Abstract

We performed angle-resolved Raman spectroscopy experiments on lead-doped and undoped Bi₂Sr₂CaCu₂O_{8+δ} (Bi2212) samples using a 660 nm laser and analyzed the Raman tensor of the phonon modes. The phonon mode was clearly observed at the 60, 103, and 630 cm⁻¹ Raman shifts. The 60, 630 cm⁻¹ peaks were only clearly observed when the incident and scattered light polarizations were configured to be parallel. The polarization angle dependence of the amplitude of the 60, 630 cm⁻¹ peak on the parallel configuration shows a twofold symmetry; therefore, both peaks originate from A_g phonons and the crystal structure of Bi2212 should be considered orthorhombic. On the other hand, the 103 cm⁻¹ peak is clearly observed in both parallel and perpendicular configurations. Remarkably, the off-diagonal component of the Raman tensor of the 103 cm⁻¹ peak showed an anti-symmetry that could not be realized within the known crystal structure of Bi2212. The implications of our findings are discussed.

Keywords: Bi₂Sr₂CaCu₂O_{8+δ}, angle resolved Raman spectroscopy, Raman tensor, orthorhombic structure, anti-symmetric off-diagonal elements

1. 서 론

지금까지 초전도 현상을 이해하기 위해 구리산화물 계열 고온 초전도체인 Bi₂Sr₂CaCu₂O_{8+δ} (Bi2212)에 대한 물성 분석이 이루어졌다. 라만 분광법 역시 많이 수행되었으며, 격자 진동인 포논에 대한 이해를 높일 수 있었다. 이러한 라만 분광법을 이용한 실험 기법 중에는 산란광에 대해 편광 방향이 입사광과 평행 혹은 수직인 것을 구분하여 얻는 것이 있다. 편광 관계에 따른 각각의 라만 반응은 입사광의 편광과 시료의 특정 축이 이루는 각도에 의해 차이가 생긴다. 이를 통해 대칭성을 분석하고 피크를 구별할 수 있다. 기존에는 시료를 직접 돌리는 방식으로 연구를 하였으나, 같은 지점을 목표하기 어렵다는 문제가 있었다. 하지만 광학계의 발달로 시료의 같은 지점에 간접적인 방식으로 각도에 대한 실험을 할 수 있게 되었고 라만 분광법에 새로운 방향성이 열리게 되었다. 본 연구에서는 각분해 라만 분광법을 통해 Bi2212의 60, 103, 630 cm⁻¹ 피크에 대한 각도에 따른 세기 및 라만 행렬을 분석하였다.

Bi2212 면 내 두 변 a, b의 길이는 5.399Å, 5.414Å이며, 사방정계 구조를 가진다고 보고되었다 [1]. 하지만 이후 몇몇 연구들에서는 정방정계로 해석해도 좋을 정도로 그 차이가 작다고 분석하였다 [2,3]. 그렇기 때문에 기존의 라만 분석에서 Bi2212의 결정 구조에 따른 포논의 구분은 D_{2h}, 혹은 D_{4h} 대칭을 기준으로 분류되었다 [4-6]. 본 연구에서는 60 cm⁻¹ 피크의 라만 행렬 분석에서 비등방성이 관측되었다. 이는 D_{4h} 대칭에서는 나올 수 없기 때문에 라만 분광법에서 Bi2212의 구조에 의한 물성은 D_{2h}로 분석해야 함을 알 수 있었다. 그리고

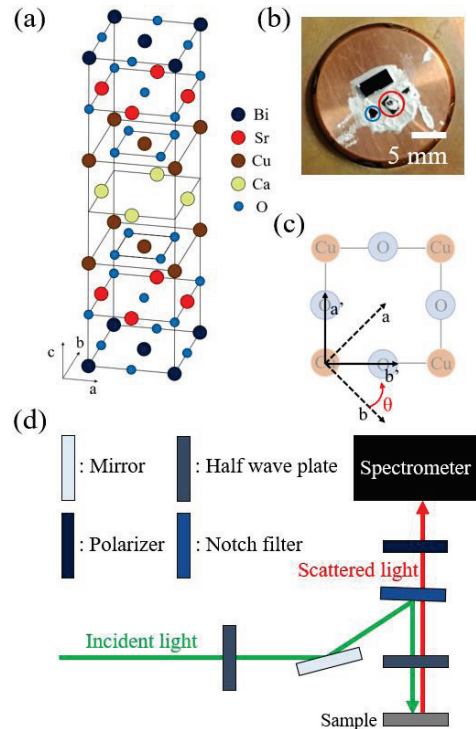


Fig. 1. (a) Crystal structure of a half unit cell of Bi2212. (b) Photograph of the sample used in this study. Red and blue circles represent Pb-doped and -doped Bi2212, respectively. (c) CuO₂-plane schematic of Bi2212 and definition of the angles used in this polarization-resolved Raman. (d) Schematic of the angle-resolved Raman spectroscopy setup.

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Continuous Template Growth of Large-Scale Tellurene Films on 1T'-MoTe₂

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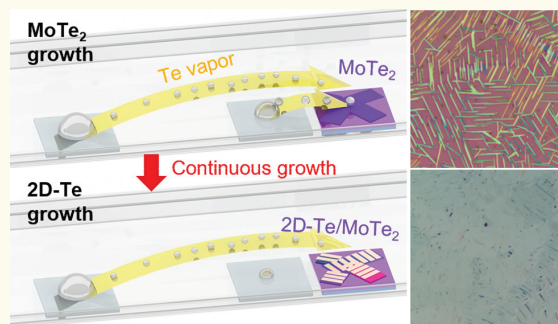
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Supporting Information

ABSTRACT: Use of a template triggers an epitaxial interaction with the depositing material during synthesis. Recent studies have demonstrated that two-dimensional tellurium (tellurene) can be directionally oriented when grown on transition metal dichalcogenide (TMD) templates. Specifically, employing a T-phase TMD, such as WTe₂, restricts the growth direction even further due to its anisotropic nature, which allows for the synthesis of well-oriented tellurene films. Despite this, producing large-area epitaxial films still remains a significant challenge. Here, we report the continuous synthesis of a 1T'-MoTe₂ template via chemical vapor deposition and tellurene via vapor transport. The interaction between helical Te and the 1T'-MoTe₂ template facilitates the Te chains to collapse into ribbon shapes, enhancing lateral growth at a rate approximately 6 times higher than in the vertical direction, as confirmed by scanning electron microscopy and atomic force microscopy. Interestingly, despite the predominance of the lateral growth, cross-sectional transmission electron microscopy analysis of the tellurene ribbons revealed a consistent 60-degree incline at the edges. This suggests that the edges of the tellurene ribbons, where they contact the template surface, are favorable sites for additional Te absorption, which then stacks along the incline angle to expand. Furthermore, controlling the synthesis temperature, duration, and preheating time has facilitated the successful synthesis of tellurene films. The resultant tellurene exhibited hole mobility as high as ~400 cm²/V s. After removing the underlying metallic template with plasma treatment, the film showed a current on/off ratio of ~10³. This ratio was confirmed by two-terminal field-effect transistor measurements and supported by near-field terahertz (THz) spectroscopy mapping.

KEYWORDS: 2D tellurium, tellurene, 1T'-MoTe₂, chemical vapor deposition, 2D materials



The rapid advancement of research in two-dimensional (2D) van der Waals (vdW)-layered materials has unveiled a wide spectrum of materials with diverse physical properties. Beginning with studies on graphene and boron nitride, the research field has expanded to numerous vdW-layered materials such as transition metal dichalcogenides (TMDs), elemental 2D layers, and combinations of various 2D components—heterostructures. Two-dimensional tellurium (2D Te), or its extended nomenclature—tellurene,¹ recently stands out as a component of the elemental 2D family, composed of stacked Te helical chains. It offers exceptional environmental stability,^{2,3} and its band gap varies with its thickness, ranging from 0.31 (bulk) to 1.17 eV (bilayer).^{2,3} Inheriting the intrinsic structure of its bulk form, current research on tellurene not only explores fascinating physics associated with topology^{4–9} but also expands into practical applications such as thermoelectric^{10–12} and piezoelectric

devices^{12,13} and flexible electronics.^{14–16} With its distinctive electrical properties and p-type characteristics,^{3,17} tellurene emerges as a potential candidate for upcoming electronics and optoelectronics applications.^{3,18–27} This has driven research into pairing it with n-type materials, leading to the development of heterostructure devices.^{27–30}

The quasi-1D helical Te chain inherently forms a hexagonal packed bundle where each chain is isotropically bound by vdW force, posing a challenge to create a 2D film. This limitation

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Viscoelastic-Support-Layer-Based Macroscopic Conformal Transfer of van der Waals Materials Compatible with Plasmonic Application

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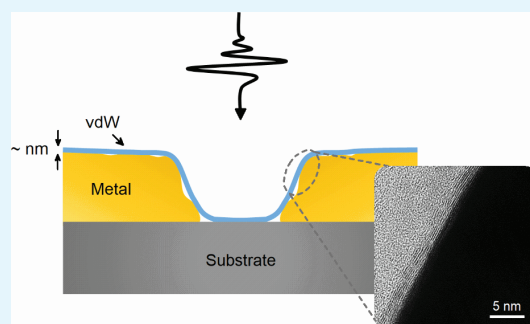
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Supporting Information

ABSTRACT: This study showcases the conformal geometries of van der Waals materials with metallic structures utilizing viscoelastic support layers. Mechanically exfoliated nanometer-thick graphite flakes were transferred onto metal structures with various side slopes using two different polymers: polycarbonate (PC) and polyethylene (PE). We proposed a morphology-based evaluation of the macroscale conformity that can contribute to the selection of a proper support layer. Although both support layers ensured high conformity on the sloped side, the PE layer offered superior conformity on the vertical metal structure. To further investigate the impact of conformal structures, we compared the terahertz transmission changes of a metal bowtie antenna before and after transferring graphite onto the bowtie gap for two distinct conformal structures. The conformity of graphite to the metal gap structure significantly influenced the optical response in the terahertz frequency regime. This suggests that the conformal structuring technique can be leveraged in various terahertz devices composed of metals and van der Waals materials, opening avenues for quantitative analysis in light–matter interactions.

KEYWORDS: viscoelastic support layer, van der Waals materials, conformal transfer, light–matter interaction, terahertz spectroscopy



1. INTRODUCTION

In addition to exploring new materials (or combinations of distinct constituents), demonstrating electronic (optoelectronic) and optical applications, and investigating novel physical phenomena,^{1–5} studying contact between metal structures and two-dimensional (2D) van der Waals (vdW) materials can significantly impact the device performance in terms of metasurfaces and metal electrode for optical and electronic applications, respectively. Metallic metasurfaces, in particular offer strong localization and enhancement of electromagnetic (EM) fields, enabling enhanced light–matter interactions beyond the diffraction limit for optical applications.^{6–11} Based on the improved device performance, studies have been conducted on 2D vdW materials combined with metal structures.^{12–16}

In order to obtain high-quality contact performance between these materials, conformal geometry between vdW materials and the metal structure can be a proper approach, where transferring vdW materials with flexible and stretchable support layers ensures high conformity to the target substrate. Conformity can be achieved on both atomic and macro scales using low glass-transition temperature (T_g) polymers.^{17–20} This advancement has led to significant improvements in device performance, particularly in terms of mobility²⁰ and is applicable to the

designed metal structure. As a result, versatile physical properties in electronic, optical, and magnetic vdW materials can be investigated by harnessing the conformal geometry.

High conformity based on the vdW materials is particularly crucial in metal gap structures, such as metal–insulator–metal (MIM) configurations,^{21,22} because the strong confinement and enhancement of EM waves in MIM structures^{23–25} allow for the investigation of nonlinear-related phenomena and plasmonic coupling beyond the diffraction limit.^{21,22,26–29} However, the seminal approaches using nanoparticle assembly or chemical vapor deposition have limitations in terms of optical parameters, materials properties, and production process. In light of these drawbacks, viscoelastic-polymer-assisted conformal transfer of mechanically exfoliated vdW materials emerges as a promising alternative approach to overcome the limitations because wide

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Understanding the oxidation chemistry of $\text{Ti}_3\text{C}_2\text{T}_x$ (MXene) sheets and their catalytic performances

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ABSTRACT

Transition metal carbides and nitrides (MXenes) nanosheets are attractive two-dimensional (2D) materials, but they suffer from oxidation/degradation issues during storage and/or applications due to their sensitivity to water and oxygen. Despite the great research progress, the exact oxidation kinetics of $\text{Ti}_3\text{C}_2\text{T}_x$ (MXene) and their final products after oxidation are not fully understood. Herein, we systematically tracked the oxidation process of few-layer $\text{Ti}_3\text{C}_2\text{T}_x$ nanosheets in an aqueous solution at room temperature over several weeks. We also studied the oxidation effects on the electrocatalytic properties of $\text{Ti}_3\text{C}_2\text{T}_x$ for hydrogen evolution reaction and found that the overpotential to achieve a current density of 10 mA cm^{-2} increases from 0.435 to 0.877 V after three weeks of degradation, followed by improvement to stabilized values of around 0.40 V after eight weeks. These results suggest that severely oxidized MXene could be a promising candidate for designing efficient catalysts. According to our detailed experimental characterization and theoretical calculations, unlike previous studies, black titanium oxide is formed as the final product in addition to white Ti (IV) oxide and disordered carbons after the complete oxidation of $\text{Ti}_3\text{C}_2\text{T}_x$. This work presents significant advancements in better understanding of 2D $\text{Ti}_3\text{C}_2\text{T}_x$ (MXene) oxidation and enhances the prospects of this material for various applications.

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1. Introduction

Two-dimensional (2D) layered structures are a class of new materials that exhibit exciting prospects for various applications [1,2]. A classic example is graphene with atomically thin sheets of carbon structurally arranged like chicken wire [3]. Meanwhile, transition metal dichalcogenides (TMDs) are semiconductors with a direct bandgap due to quantum confinement [4,5]. Furthermore, elemental 2D materials such as phosphorene and antimonene possess highly tunable bandgaps just by adjusting the number of layers, making them attractive for various applications [6]. In 2011, transition metal carbides, carbonitrides, and nitrides with the gen-

eral formula $\text{M}_{n+1}\text{X}_n\text{T}_x$ (M represents transition metals such as Ti, V, Nb, and Mo; X is C and/or N, T_x expresses termination groups such as $-\text{OH}$, $=\text{O}$, and $-\text{F}$) have been introduced as MXene to the family of 2D materials [7]. In general, MXenes are synthesized by a wet-chemical method from ceramic $\text{M}_{n+1}\text{AX}_n$ phases (MAX, A is an A-group element such as Al, Si, Ga, etc.) [7,8].

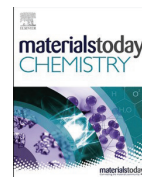
$\text{Ti}_3\text{C}_2\text{T}_x$ is the most widely used MXene and exhibits attractive chemical, optical, physical, and mechanical properties including excellent conductivity, high surface area, and good optical transparency [9–14]. These fascinating properties make MXene nanosheets attractive for a wide range of applications such as energy storage [15], sensing technology [16,17], electromagnetic interference shielding [18,19], membranes [20–23], catalysis [24,25], optoelectronics [26,27], and photovoltaics [28]. MXenes are composed of stacked metallic-like ultrathin sheets held together by the termination groups, making them strongly hydro-

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Enhancing heat generation ability and magnetic properties of MgFe_2O_4 with Ni substitutes for biomedical applications

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ABSTRACT

Herein, using the systematic experimental characterization and density functional theory calculations, the effects of Ni elemental substitution on the crystal structure, cation distribution, magnetic properties, and heat generation ability of $\text{Mg}_{1-x}\text{Ni}_x\text{Fe}_2\text{O}_4$ ($x = 0, 0.2, 0.4, 0.6, 0.8, \text{ and } 1$) have been reported. We demonstrate that the presence of Ni can lead the lattice shrinkage, phase transition from partially to fully inverse spinel structure, and multi-domain structures in the samples. Moreover, Ni substitutional atoms make magnetically harder ferrite, associated with increasing magnetic inhomogeneity. Remarkably, anomalous enhancements of magnetic anisotropic and coercivity have been observed in $\text{Mg}_{0.4}\text{Ni}_{0.6}\text{Fe}_2\text{O}_4$. Heat generation ability (SAR) increases from 242 W g^{-1} for $x = 0$ – 391 W g^{-1} for $x = 0.6$ at an AC magnetic field of 370 kHz frequency. Thus, the magnetic hyperthermia of $\text{Mg}_{0.4}\text{Ni}_{0.6}\text{Fe}_2\text{O}_4$ sample on HeLa shows promising results that the viability of cells decreases to 25 % after 5 sessions. The present experimental and theoretical systematic studies provide novel avenue and paradigm for the development of ferrite nanoparticles with high heat generation ability and magnetic properties for biomedical applications.

1. Introduction

Over several decades the ferrites (of chemical formula MFe_2O_4 , $\text{M} = \text{Mg, Mn, Co, Fe, Ni, and Zn}$) have attracted tremendous attention due to their unique magnetic properties and low production costs for a wide range of applications, including magnetic recording media [1], sensors [2], photocatalysis [3], ferrofluids [4], magnetic resonance imaging [5], drug delivery [6], and biomedicine. In particular, magnesium ferrite with a structural formula of $(\text{Mg}_{1-\delta}\text{Fe}_\delta)_\text{A}[\text{Mg}_\delta\text{Fe}_{2-\delta}]_\text{B}\text{O}_4$ [inversion parameter $0 < \delta \leq 1$, a distribution of iron ions in the tetrahedral (A) and octahedral (B) sublattices] has been identified to exhibit the highest heat generation ability among the cubic spinel ferrites for the thermal coagulation therapy of cancer tumors and good biocompatibility [7–9]. Generally, an efficient method for controlling heat generation ability with modified properties involves varying the chemical composition (such as dopant elements) and inversion parameter (or ratio of

components) [10–12]. For example, the B site Cu^{2+} substitutes with $\delta \approx 0.9$ can lead to improved heat generation [9,13,14] although Cu doped MgFe_2O_4 exhibits the weak intrinsic magnetic property (with a magnetic moment of $1.9 \mu_\text{B}$ for formula unit (f.u)). Furthermore, Ni^{2+} doped MgFe_2O_4 exhibits the decent magnetic properties and highest heat generation ability compared to other spinel ferrites [7]. The divalent Ni dopant lies at the B site in bulk form and is predicted to increase the magnetic moment of the sample, which can be described by Néel's two-sublattice model [15]. According to the structural formula of the doped composite $(\text{Mg}_{1-\delta}\text{Fe}_\delta)_\text{A}[\text{Mg}_{\delta-x}\text{Ni}_x\text{Fe}_{2-\delta}]_\text{B}\text{O}_4$, the net magnetic moment can be written as $M_\text{net} = M_\text{B} - M_\text{A} = x \cdot M_\text{Ni} + M_\text{Fe}(2 - \delta)$, where M_Fe and M_Ni are the magnetic moments of the Fe^{3+} and Ni^{2+} ions at room temperature ($M_\text{Fe} = 5.9 \mu_\text{B}$ and $M_\text{Ni} = 3.2 \mu_\text{B}$), while the Mg^{2+} is a nonmagnetic ion [14].

Although there have been several studies on Ni doped ferrite compounds [15], little research has been conducted on structural evolution

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Full length article

Fe-Ni based alloys as rare-earth free gap permanent magnets

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ABSTRACT

$L1_0$ -ordered FeNi phase has potential as a rare-earth free permanent magnet due to its large magnetization and high Curie temperature. However, low long-range crystal ordering and weak magnetocrystalline anisotropy (K_u) impede its practical use in a technologically relevant permanent magnet. Employing density functional and Monte Carlo simulations, we demonstrate the substantial improvements on structural and thermal stability, disorder-order phase transition, and intrinsic permanent magnetic properties of Fe-Ni structures. We propose that this is achievable through the complementary elemental substitutions by metal elements (M) and interstitial doping with 2p elements. $\text{Fe}_{1-x}\text{M}_x\text{Ni}$ with simple metal ($\text{M} = \text{Ga}$ and Al) and metalloid ($\text{M} = \text{Ge}$ and Si) elemental substitutions at $x = 0.5$ are broadly known as Heusler ($L2_1$) structures with no permanent magnet characteristics. On the contrary, we find that for $x = 0-0.5$, $L1_0$ -type structure is energetically favored over the Heusler-type structures. The predicted structures exhibit K_u values up to approximately 2.1 MJ/m^3 , which is roughly three times that of FeNi phase (0.68 MJ/m^3), where the absolute value of K_u depends on the degree of $L1_0$ crystal ordering. Interstitial doping with B elevates K_u further up to a maximum value of 3.9 MJ/m^3 (at 0 K), leading to room-temperature intrinsic permanent magnetic properties, including maximum energy density product $(BH)_{\text{max}}$, anisotropy field $\mu_0 H_a$, and hardness parameter κ , comparable to the widely investigated MnBi and MnAl permanent magnets. These results may serve as a guideline in designing Fe-Ni based rare-earth free gap permanent magnetic materials that were previously overlooked.

1. Introduction

Permanent magnets are the key components in electric motors, wind turbines, actuators, data storage memories, and semiconductor devices [1–5]. Currently, high-performance permanent magnets are made of as a combination of 3d magnetic transition metal (TM) and rare-earth (RE) 4f elements [6–13]. Neodymium-iron-boron ($\text{Nd}_2\text{Fe}_{14}\text{B}$) provides the highest-performing permanent magnetic properties at present [14–19]; maximum energy density product $(BH)_{\text{max}}$ of $360-400 \text{ kJ/m}^3$ [16–18], uniaxial magnetocrystalline anisotropy K_u of 4.5 MJ/m^3 [15], and saturation magnetization $\mu_0 M_s$ of $1.57-1.61 \text{ T}$ at room temperature [14,15]. However, the $(BH)_{\text{max}}$ value of $\text{Nd}_2\text{Fe}_{14}\text{B}$ magnet falls very steeply above room temperature [18,19], accompanied with a low Curie temperature (T_C) of 585 K [15]. Therefore, practical use of $\text{Nd}_2\text{Fe}_{14}\text{B}$ in high-temperature permanent magnet applications is limited. In addition, Nd is a relatively expensive element.

As yet, reducing the amount of RE in RE 4f-TM 3d compounds, or complete elimination if possible, without much sacrificing their performance has been the focus of attention [6–13]. In particular, the so-called gap permanent magnets, whose $(BH)_{\text{max}}$ is in between ferrites

$\text{Ba}(\text{Sr})\text{Fe}_{12}\text{O}_{19}$ ($11.9-37.4 \text{ kJ/m}^3$) [20–22] and $\text{Nd}_2\text{Fe}_{14}\text{B}$ ($360-400 \text{ kJ/m}^3$) [16–18], are called for as substitute for RE permanent magnets. Among the potential gap magnets, Fe-Ni alloys have received considerable attention thanks to their large $\mu_0 M_s$ of $1.5-1.72 \text{ T}$ [23–30] and high T_C of $647-873 \text{ K}$ [25–29]. These values are higher than those of the widely investigated gap permanent magnets such as MnAl (0.82 T and $558-646 \text{ K}$) [31–33] and MnBi ($0.8-0.93 \text{ T}$ and $628-635 \text{ K}$) [34–36]. However, the low long-range ordering parameters ($0.33-0.61$) of the alternating Fe and Ni layers [37–39] observed in FeNi, which is quite difficult to control in a sample preparation, cause a quick degradation of intrinsic hard magnetic properties. Numerous efforts have been made to improve the structural stability and ordering parameter of FeNi crystals [40–43]. Relatively high ordering parameters of up to $0.71-0.87$ have been reported albeit in a thin-film limit [40–43]. Recently, the highly ordered $L1_0$ crystals of bulk Fe-Ni phase have been produced when up to 13 at.% P and 7 at.% C are present in Fe-Ni samples [44]. These findings suggest feasibility of the $L1_0$ -ordered Fe-Ni crystals with appropriate alloying compositions. Another major shortcoming of FeNi magnet is its low K_u , $0.55-1.3 \text{ MJ/m}^3$ [23–30,37–43], which results in coercivity (H_c) and $(BH)_{\text{max}}$ that are insufficient

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Intrinsic magnetism of an individual rare-earth atom on transition metal dichalcogenide semiconductors

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ABSTRACT

Two-dimensional (2D) structures that exhibit intriguing magnetic phenomena such as perpendicular magnetocrystalline anisotropy (PMA) have become a focus of spintronic research due to their potentials in maximizing the information storage density. Herein we perform density-functional theory plus U (DFT+U) calculations to investigate the binding affinity and intrinsic magnetic properties of an individual rare-earth (RE) Sm atom on WSe₂ monolayer. Our calculations show that Sm adatom energetically prefers to adsorb at the W-top site in WSe₂ rather than the Se-top and hollow sites. We predict extremely large PMA values of ~7–33 meV per Sm at the most stable W-top site, depending on U parameter in DFT+U calculations, while it is negligibly small for the Se-top and hollow sites. The underlying mechanism for large PMA is elucidated in terms of the strong spin–orbit coupled Sm 4*f* – W 5*d* orbital states and large 4*f* orbital magnetic moment in the high-spin crystal field. These results provide a viable route to achieving an atomic scale *f*-electron PMA in 2D structures, opening interesting prospects in two-dimensional semiconducting spintronics.

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I. INTRODUCTION

Over the years after the discovery of graphene,¹ the exploration of two-dimensional (2D) transition metal dichalcogenides (TMDs) has been active in materials science, owing to their unique atomic and electronic structures. In particular, researchers focus on engineering the physical and chemical properties of TMD MX₂, where M is a transition metal element (M = Mo or W) and X is a sulfur element (X = S or Se), by decorating with functional species or/and metal dopant atoms.^{2–5} Nevertheless, rare-earth (RE) elements have not been seriously considered as dopants, leaving unanswered questions what would be the effect of RE dopant atoms on the physical properties of MX₂ monolayers. Recently, MoS₂ monolayer with Sm dopant atoms has been successfully grown by chemical vapor deposition method.⁶ The authors reported that the presence of Sm substantially alters the semiconducting electronic properties of MoS₂ monolayer. Furthermore, there have been few more experimental and theoretical studies on transition metal (TM) and RE doped MX₂

monolayers.^{7–9} For instance, the possible magnetic nature of Sm-doped MoS₂ monolayer has been predicted by the density-functional theory (DFT) calculations, which is attributed to the hybridization between the Sm-4*f* and S-3*p* orbital states.⁸ It has also been experimentally reported that the V-doped WSe₂ structures exhibit magnetic ordering at room temperature.^{10,11} In addition to these remarkable findings, the promising alternative seemingly resides in the adoption of the strong spin–orbit coupling (SOC) features of *f* and *d* electrons for exploring the magnetocrystalline anisotropy (MA). If ever realized, large perpendicular MA (PMA) is of crucial significance for stable long-term spintronic applications.

In this paper, we perform the DFT plus U (DFT+U) calculations to investigate the binding affinity and intrinsic magnetic properties of an individual Sm atom on WSe₂ monolayer. Our calculations show that Sm adatom energetically prefers to adsorb at the W-top site rather than the Se-top and hollow sites. We predict extremely large MA energy (MAE) values of 7–33 meV per Sm adsorbed at the most stable W-top site in WSe₂ monolayer,

Optical Tweezing Terahertz Probing for a Single Metal Nanoparticle

Jiyeah Rhie,[#] Dukhyung Lee,[#] Taehoon Kim, Seonghun Kim, Minah Seo, Dai-Sik Kim,^{*} and Young-Mi Bahk^{*}



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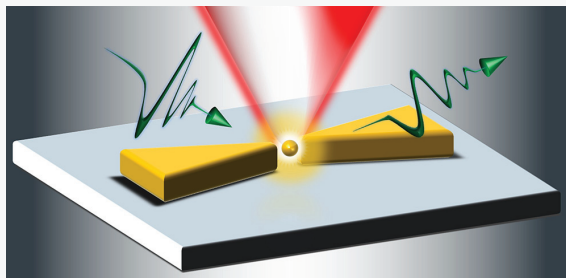
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Supporting Information

ABSTRACT: Recently, extensive research has been reported on the detection of metal nanoparticles using terahertz waves, due to their potential for efficient and nondestructive detection of chemical and biological samples without labeling. Resonant terahertz nanoantennas can be used to detect a small amount of molecules whose vibrational modes are in the terahertz frequency range with high sensitivity. However, the positioning of target molecules is critical to obtaining a reasonable signal because the field distribution is inhomogeneous over the antenna structure. Here, we combine an optical tweezing technique and terahertz spectroscopy based on nanoplasmonics, resulting in extensive controllable tweezing and sensitive detection at the same time. We observed optical tweezing of a gold nanoparticle and detected it with terahertz waves by using a single bowtie nanoantenna. Furthermore, the calculations confirm that molecular fingerprinting is possible by using our technique. This study will be a prestep of biomolecular detection using gold nanoparticles in terahertz spectroscopy.

KEYWORDS: Terahertz spectroscopy, single particle detection, optical trapping, bowtie antenna, molecular fingerprinting



Gold nanoparticles (NPs) have been studied in spectroscopic research fields with great attention to their unique optical and electric properties due to their capabilities in tracking and drug delivery,¹ catalysis,² cancer therapy,³ chemical and biological sensing,⁴ optoelectronics, and biological imaging.⁵ Terahertz studies have also shown interest in detecting gold NPs to boost the performance of these applications.^{6,7} This is because gold NPs can help the target molecules be more sensitively detectable, where the NPs are linked to the targets, with the fact that gold has refractive values larger than those of any other materials. By that fact, therefore, the terahertz sensing signals can be maximized with gold NPs. First, one of the advantages of the terahertz wave is the detection of molecules through resonant absorption peaks due to the molecular intrinsic and rotational/vibrational modes.^{8,9} Another advantage of using terahertz waves is that they are basically nondestructive electromagnetic waves due to their low photon energy (1 THz is 4 meV) in high repetition rate systems (80 MHz pulse laser-based system).¹⁰ Therefore, biorelated nanomaterials involving cells and molecules can be detected without thermal damage or ionization effect, as compared to the system using near-infrared or visible light.^{11,12}

However, gold NPs themselves are difficult to observe in the terahertz spectrum due to their extremely subwavelength size, which results in weak light absorption and scattering.¹³ To overcome these limitations, plasmonic nanoantenna structures, including nanopillars,¹⁴ nanoholes,¹⁵ diabolos nanoantennas,¹⁶ and slot nanoantennas,^{17–22} have been proposed as excellent

tools that strongly localize electromagnetic fields into nano-scale volume. For instance, it has been reported that when a single 100-nm-sized gold particle is placed at the center of a slot antenna with a relevant size of nanogaps (120 nm-wide gap), a remarkable decrease in terahertz transmission occurs.²² This means that a single gold NP can be detected with terahertz waves having several hundred micrometers to millimeters wavelengths. This is due to the strongly enhanced interaction between the localized terahertz electric field and a gold NP. Furthermore, biomolecular detection has recently been observed in ring-shaped slot antenna arrays with a gap size of a few nanometer scales.²³ Although it has been experimentally and theoretically demonstrated in previous studies that single gold NP detection is possible with terahertz waves, it still significantly requires further positioning techniques of target samples freely.

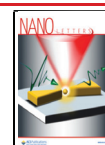
Here, we combined a reflection-type terahertz time-domain spectroscopy (THz-TDS) system with an optical tweezing system to precisely locate a single gold NP, where terahertz waves are strongly focused by a bowtie antenna structure. This

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In-depth study for confinement of electromagnetic wave in a coupled terahertz nano-slot antenna

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Abstract

We study the electric-field confinement of terahertz waves in an asterisk-shaped nano-slot antenna using finite-element-method-based simulations. The asterisk slot antenna, consisting of a single slot and an X-shaped slot antenna, exhibits near- and far-field characteristics depending on the coupling angle. In a regime of small coupling angles, three-dimensional confinement of terahertz waves is realized in X-shaped and coupled slot antennas. We analyze the coupling-angle-dependent confinement performance in terms of localized field strength and focusing degree, considering the gap size of the slot antennas. These findings provide valuable design insights for studying light-matter interactions in the terahertz frequency regime.

Keywords Terahertz spectroscopy · Finite element method · Metamaterials · Slot antennas

1 Introduction

Transmission of electromagnetic waves through a small hole compared to the wavelength is restricted by the diffraction limit, which can be overcome by utilizing the plasmonic effect with periodically arranged metallic hole structures [1]. Following the seminal work, it has been shown that both the shape and periodic arrangement of holes can improve transmittance properties [2–8]. These extraordinary optical transmission (EOT) phenomena have been observed across a wide frequency range, including visible, near-infrared, terahertz, and microwave. Since most metals can be assumed to be perfect electric conductors in long wavelength ranges, the EOT phenomena can be explained by the quasi-static model and wave-guide effect [9, 10]. For example, as the length-to-width ratio of a rectangular metallic hole increases, the

hole's shape, rather than its periodic arrangement, predominantly affects resonant transmission phenomena [11, 12]. The effect can be explained by the resonance of a rectangular-shaped slot antenna; even though the width of the rectangular hole and the thickness of the metal are much smaller than the wavelength of an electromagnetic wave, the resonance can take place for an electromagnetic wave with wavelength twice of the length of the slot [10, 13]. The unexpectedly high transmittance can be attributed to strong confinement and enhancement of electromagnetic waves in the metallic hole.

In the case of metallic nano-gap structures such as nano-slit or nano-slot antenna with nm-scale width, an incident electromagnetic wave with a long wavelength, such as terahertz waves, induces current inside a metal film. As a result, charges accumulate at the gap edges, and the entire system can be modeled as an electrical circuit in terms of a capacitor. The capacitive coupling of two metal edges leads to strong electric-field enhancement inside and near the gap [10, 14]. As the gap size decreases from a few hundred nanometers to a few nanometers, the confinement and enhancement of the electric field increases, which can be explained by the quasi-static capacitance model [15, 16]. By overcoming the diffraction limit through the confinement and enhancement of the electromagnetic waves using metal gap structures, strong light-matter interaction is feasible, and extensive research on materials properties, bio-sensing, and terahertz nonlinear optics are expected [17–25].

Taehoon Kim, Seonghun Kim, and Sung Ju Hong have contributed equally to this work.

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Bridging terahertz metal slot antenna with parameterized electrical conductivity: insights from finite element method and microscopic theory

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Abstract

Metal slot antennas exhibit high transmission characteristics at resonant frequencies when electromagnetic waves with polarization in the width direction of the rectangular hole structure enter, having wavelengths approximately twice the length of the rectangular hole. In this study, we utilize COMSOL multiphysics simulation to examine the transmission behaviors of such resonators operating in terahertz frequency range, with a specific emphasis on their performance when incorporating micron-sized conductive embedding within the central region of the rectangular slot. We observe that as the conductivity of the embedding material increases, the resonant frequency undergoes a shift towards higher values through non-resonant behaviors in the intermediate conductivity range, eventually reaching nearly twice the fundamental resonant mode. The additional analytic microscopic calculation reveals that the interference effect of the electromagnetic field inside the slot antenna can be responsible for the transmittance modifications and provides a reference for investigating unknown embedded targets. These findings provide valuable insights into the versatile applications of metal slot antennas, particularly in areas such as sensing and detection of subwavelength materials.

Keywords Terahertz frequency · Slot antennas · Electrical conductivity · COMSOL Multiphysics · Microscopic diffraction model

1 Introduction

Metal slot antennas, renowned for their ability to focus and enhance electromagnetic waves at specific frequencies within a deep subwavelength volume [1–4], are indispensable tools in diverse fields such as molecule detection and the study of low-dimensional materials, including nanoparticles and nanofilms [5–8]. These antennas, with their tailored

resonance properties, offer unique opportunities for probing materials and molecules at the nanoscale [9–12].

In recent advancements, nano slot antennas have emerged as powerful tools for detecting minute substances in the terahertz range, effectively addressing the challenge of small molecule absorption cross-section [13–15]. Furthermore, leveraging nanometer-scale thin films, researchers have developed terahertz switching devices that integrate nano-resonator arrays with thin films such as VO₂ nanofilms and graphene [16]. This innovation not only expands the spectrum bandwidth by tenfold but also allows for precise modulation of nano-resonator characteristics within the terahertz frequency range [17]. Such breakthroughs underscore the vast potential of thin film technology in enhancing the capabilities of slot antennas for various applications.

Understanding the intricacies of resonance behavior is pivotal for optimizing antenna performance. For instance, previous studies have demonstrated how connecting the central area of a slot with metal or partially filling it with nanoparticles can induce metallic nano-bridges, altering the

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Anti-ambipolar transport and logic operation in two-dimensional field-effect transistors using in-series integration of GeAs and SnS₂

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ABSTRACT

Germanium arsenide (GeAs) from the IV–V semiconductor family has drawn attention for its anisotropic optical and electrical characteristics as a robust p-type semiconductor with high mobility. Despite its potential, the scope of applications for two-dimensional (2D) GeAs remains limited. In this study, we investigate the in-series integration of GeAs and SnS₂ for complementary inverter operation, a crucial element in logic circuits. Moreover, the resulting in-series field-effect transistors reveal a previously undocumented anti-ambipolar behavior in this configuration, with a peak-to-valley ratio exceeding 5000 at a drain-source bias of 2 V. This anti-ambipolar characteristic operates regardless of the direction of current flow, which is rarely seen in conventional anti-ambipolar transistors built with hetero-stacked p–n junctions. Moreover, the in-series structure demonstrates stable operation as a complementary inverter, highlighting the versatile potential of p-type 2D GeAs in diverse optoelectronic applications. This work contributes insights into the transport characteristics of GeAs and its interconnected arrangement with SnS₂, offering valuable implications for the development of advanced 2D material-based devices.

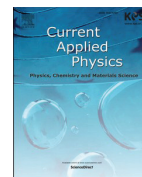
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The advent of two-dimensional (2D) layered materials has sparked significant interest in their physical properties and potential applications to nano-optoelectronic devices.^{1,2} Presently, they stand at the forefront of materials sciences, poised to lead the next generation of advanced materials due to their incomparable versatility and tunability, offering abundant possibilities for innovative applications. Extensive studies on tens of layered materials have already been documented in the literature,^{3,4} and this exploration is continuously expanding. Methodologies and innovative combinations are actively evolving, leveraging the foundation of existing layered materials. Examples include the creation of vertical p–n heterojunctions^{5,6} and Moiré superlattices,^{7,8} each designed to identify optimal applications and unveil physical phenomena. These ongoing explorations promise to unravel unprecedented insights into the intricate world of 2D layered materials.

Among the diverse landscape of 2D layered materials, germanium arsenide (GeAs) has emerged as a compelling addition, capturing attention for several reasons.^{9–23} Belonging to the IV–V semiconductor family, GeAs is distinguished by exhibiting a pronounced anisotropy in optical and electrical properties,²³ stemming from its anisotropic

crystal structure. This characteristic yields a polarization-sensitive photo-response and anisotropic mobility.^{22,23} Its bandgap size follows a general trend, increasing as the thickness decreases. However, even in its monolayer form, GeAs retains an indirect bandgap of 2.06 eV,¹⁷ setting it apart from the majority of layered semiconducting materials. Moreover, few-layer GeAs transistors have demonstrated remarkable maximum mobility of $\sim 100 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ and on/off ratio of $\sim 10^5$ with a distinct p-type behavior.²² This air-stable p-type character, coupled with high mobility, represents a significant advantage in optoelectronic applications. In addition, GeAs gains its significance not only from the scarcity of p-type layered materials²⁴ but also from the challenges associated with achieving p-type doping,²⁵ highlighting its crucial role in this field.

Despite these promising optical and electrical properties, the exploration of applications for 2D GeAs remains relatively limited, even with the passage of several years since the initial demonstration of a few layered GeAs transistors.²² While 2D GeAs is an excellent material for transistors and photodetectors on its own, its optimal application is expected to arise when combined with other 2D layered materials, as this approach constitutes the essence of layered material



First-principles study of the thickness-dependent shift current in γ -GeSe thin layers

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ABSTRACT

We investigate the stable structure and optoelectronic properties of thin γ -GeSe layers through first-principles calculations. We examine three stacking configurations (A-A, A-B, and A-C) of adjacent quadruple layers (QLs), revealing the structural stability of A-C stacking. Due to broken inversion symmetry in atomically thin γ -GeSe layers, shift currents are generated, which are very sensitive to their stacking order and thickness. Despite similar optical absorption trends in A-B and A-C stackings, their shift current responses differ significantly. The shift current is notably decreased at odd-number stackings for all cases, attributed to opposite generated flows between the top and bottom surfaces. The analysis of orbital contributions reveals the charge shift's origin in γ -GeSe. We also explore mechanical modifications, such as sliding and strain, demonstrating the tunability of the shift current spectrum in γ -GeSe. This research enhances our understanding of the optoelectronic response in atomically thin materials, providing valuable insights for future applications.

1. Introduction

Recently, a novel γ phase of group-IV monochalcogenides has been experimentally synthesized, demonstrating superior conducting behavior compared to graphite [1]. The potential existence of the γ phase in a two-dimensional limit was predicted through first-principles calculations [2], and it has been successfully realized via chemical vapor deposition synthesis. The lack of inversion symmetry results in electric polarization in γ -GeSe, which is sensitive to uniaxial and biaxial strains [3]. A constituent quadruple layer (QL) of γ -GeSe possesses inversion symmetry, but its stacking along the z -axis induces non-vanishing polarization, consequently being closely related to the stacking order [4].

In noncentrosymmetric materials, uncompensated photocurrent can spontaneously develop when external light is incident. In particular, shift current, also known as the bulk photovoltaic effect, can be generated due to the second-order nonlinear response to linearly polarized light [5–9]. In the bulk of γ -GeSe, the presence of shift current has been investigated; the shift current varies depending on external strain and resultant band inversion at the Γ point [10]. However, whether shift current is generated in atomistically thin limits and how it is related to the stacking order and thickness remain elusive. Given the establishment of large shift currents in low-dimensional systems [11–13], an investigation into the relationship between the stacking of QLs and the

development of shift current is imperative.

In this study, we explored the variation of shift current with different stacking orders in γ -GeSe using first-principles calculation. We found that the generated shift current is sensitive to the stacking configurations of γ -GeSe. Additionally, we identified the origin of the reduced shift current at odd-numbered QLs and underscored the significance of the inter-QL interaction in shift current generation. Furthermore, mechanical modifications, such as sliding and strain, were also suggested as potential methods for controlling and tuning the shift current spectrum in γ -GeSe. Overall, our findings contribute to a more profound understanding of the optoelectronic behavior in atomically thin nanostructures.

2. Methods

Our density functional theory (DFT) calculations were performed with the projected augmented plane-wave method [14], implemented in the Vienna Ab initio Simulation Package (VASP). The screened hybrid functional of Heyd, Scuseria, Ernzerhof (HSE) [15] was applied to accurately capture the insulating bandgap of γ -GeSe [16,17]. The cutoff energy for the plane-wave-basis expansion was set to 500 eV. For Brillouin zone sampling, we used $12 \times 12 \times 3$ k-point grids for the bulk phase and $12 \times 12 \times 1$ k-point grids for the two-dimensional thin layers.

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Non-halogenated and non-volatile solid additive for improving the efficiency and stability of organic solar cells†

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Various high boiling point solvent additives are commonly used for high-performing organic solar cells (OSCs). However, they are not only usually detrimental to device stability but also often contain halogens, rendering them highly reactive and environmentally unfriendly. Herein, a non-halogenated and non-volatile solid additive, 2-(4-phenoxybenzylidene)-1*H*-indene-1,3(2*H*)-dione (PID), is synthesized for the preparation of high-performing and highly stable non-fullerene acceptor (NFA)-based OSCs. PID introduction enhances the molecular ordering of the photoactive materials and optimizes their nanoscale phase-separated morphology by controlling intermolecular interactions. The power conversion efficiency (PCE) of the resulting PID-processed OSC devices increases from 13.9% to 16.3%, surpassing that of 1-chloronaphthalene (CN)-processed devices (15.5%). Moreover, when burn-in losses are eliminated, the PID-processed devices demonstrate excellent thermal stability, retaining 98% of their initial PCE after 1200 h at 85 °C. Such high thermal stability renders these devices among the most stable PM6:Y6-based OSCs reported to date. The results suggest that non-volatile solid additives can simultaneously enhance the efficiency and stability of OSC devices without halogens, thereby opening up possibilities for their future commercial applications.

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Introduction

Organic solar cells (OSCs) have garnered significant attention owing to their lightweight, low cost, high flexibility, and simple device structure.^{1–3} In bulk heterojunction (BHJ) OSCs, essential photoelectric conversion processes, including charge generation, transport, and recombination, are closely related to the morphology of the active layer, which consists of a blend of donor and acceptor molecules. Therefore, an ideal morphology with high crystallinity and a proper domain size is crucial for

achieving high power conversion efficiencies (PCEs) and potential stability in OSCs.^{4,5}

Several methodologies, such as thermal annealing (TA),⁶ vapor annealing,⁷ and processing additives,^{8,9} have been developed to achieve a fine morphology with optimal nanoscale phase separation. Among these techniques, the introduction of additives, such as 1,8-diiodooctane (DIO),^{10,11} 1-chloronaphthalene (CN),^{12,13} and diphenyl ether (DPE),^{14,15} into the host solvent has been widely employed to optimize the nano-morphology of the active layer because of its facile and efficient processability. The presence of solvent additives with a high boiling point and selective solubility reduces the over-aggregation of small-molecule acceptors in photoactive films, resulting in a favorable BHJ morphology.^{16,17} However, despite their effectiveness in improving the performance of OSC devices, less volatile additive molecules tend to remain within the active layer, potentially serving as pathways for OSC degradation.¹⁸ BHJ layers containing high boiling point processing additives are susceptible to undesirable morphological evolution, as the residual solvents slowly yet continuously change the BHJ morphology, thus compromising device stability.^{19–22} Additionally, a halogenated solvent additive that is light-sensitive and forms reactive radicals can initiate and accelerate the degradation of photoactive materials, further compromising the photostability of OSC devices.^{20,23}

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Understanding of Defect Passivation Effect on Wide Band Gap p-i-n Perovskite Solar Cell

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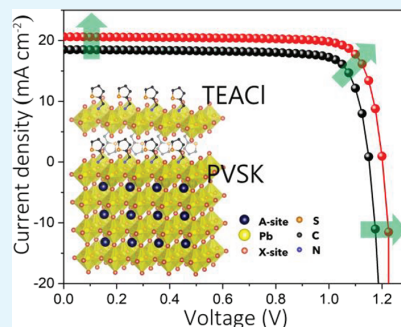
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Supporting Information

ABSTRACT: The wide band gap perovskite solar cells (PSCs) have attracted considerable attention for their great potential as top cells in high efficiency tandem cell application. However, the photovoltaic performance and stability of PSCs are constrained by nonradiative recombination, primarily stemming from defects within the bulk and at the interface of charge transport layer/perovskite and phase segregation. In this study, we systematically investigated the effects of 2-thiophenethylammonium chloride (TEACl) on a wide band gap (~ 1.67 eV) $\text{Cs}_{0.15}\text{FA}_{0.65}\text{MA}_{0.20}\text{Pb}(\text{I}_{0.8}\text{Br}_{0.2})_3$ (CsFAMA) perovskite solar cell. TEACl was employed as a passivation layer between the perovskite and electron transport layer (ETL). With TEACl treatment, charged defects responsible for sub-band absorption and electrostatic potential fluctuation were effectively suppressed by the passivation of bulk defects. The incorporation of TEACl, which led to the formation of a TEA_2PbX_4 /Perovskite (2D/3D) heterojunction, facilitated better band alignment and effective passivation of interface defects at the ETL/CsFAMA. Owing to these beneficial effects, the TEACl passivated PSC achieved a photo conversion efficiency (PCE) of 19.70% and retained $\sim 85\%$ of initial PCE over ~ 1900 h, surpassing the performance of the untreated PSC, which exhibited a PCE of 16.69% and retained only $\sim 37\%$ of its initial PCE.

KEYWORDS: perovskite solar cell, wide band gap perovskite, p-i-n structure, 2D/3D heterojunction, defect passivation



1. INTRODUCTION

Metal halide perovskite solar cells (PSCs) have attained huge attention over the past decade because of their high crystallinity,¹ high absorption coefficient (10^4 – 10^5 cm^{-1}),^{2,3} low exciton binding energy,^{4,5} higher charge-carrier mobility,^{6,7} and band gap tunability (1.1–2.3 eV).^{8–10} Even though the first ever reported power conversion efficiency (PCE) of PSCs was 3.8% in 2009, the PCE has skyrocketed to 26.1% by 2023 due to rapid development and engineering.^{11–13} However, achieving over 33% of PCE with a single-junction solar cell is theoretically unattainable due to the Shockley–Queisser limit (SQ limit).¹⁴ Thus, tandem solar cells could be a solution to overcome this limit by combining narrow- and wide-band gap (WBG) junctions. The WBG perovskite solar cells with bandgaps greater than 1.67 eV have achieved PCE over 20%, proving crucial for mitigating energy losses in single-junction devices attributable to unabsorbed photon energy and thermalization.¹⁵ As of now, WBG (>1.67 eV) PSCs, achieving PCE over $\sim 20\%$, stand out among polycrystalline thin-film solar cells, positioning them as promising candidates for top cell application in tandem solar cell.^{16–19} However, WBG PSCs suffer from a significant loss in open circuit voltage (V_{OC}), a parameter crucial for reaching their theoretical limit.^{18,20,21} The substantial deficit in V_{OC} mainly resulted from nonradiative recombination. Nonradiative recombination in

perovskite thin films is primarily influenced by two factors: deep-level trap states within the bulk and at the interface, as well as energy-level disparities between the perovskite and the charge transport layers (CTL).^{17,22–27} Notably, the deep-level trap states at the interface (CTL/Perovskite) have a major role on nonradiative recombination due to its higher defect densities.^{26,28} To counter surface-related nonradiative recombination at the interface (C_{60} /Perovskite), various strategies have been employed. These include applying a MgF_x (~ 1 nm) interlayer to adjust surface energy²⁹ and intentionally forming 2D or lower-dimensional perovskite^{30,31} to mitigate losses in V_{OC} . Chen et al. demonstrated the formation of 2D/3D heterojunction interface by using 2-thiophenethylammonium chloride (TEACl) and achieved average PCE 19.50% for optimized condition due to interface passivation.¹⁶

In this study, we systematically investigated the passivation effects of TEACl on WBG triple-cation perovskite, $\text{Cs}_{0.15}\text{FA}_{0.65}\text{MA}_{0.20}\text{Pb}(\text{I}_{0.8}\text{Br}_{0.2})_3$ (CsFAMA), by comprehen-

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Naphthalene Diimide-Modified SnO₂ Enabling Low-Temperature Processing for Efficient ITO-Free Flexible Perovskite Solar Cells

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A low-cost and indium-tin-oxide (ITO)-free electrode-based flexible perovskite solar cell (PSC) that can be fabricated by roll-to-roll processing shall be developed for successful commercialization. High processing temperatures present a challenge for the PSC fabrication on flexible substrates. The most efficient planar n-i-p PSC structures, which utilize a metal oxide as an electron transport layer (ETL), necessitate high annealing temperatures. In addition, the device performance deteriorates owing to the migration of halogen ions, which causes the oxidation of the metal electrodes. These drawbacks conflict with the development of highly efficient flexible PSCs fabricated on ITO-free transparent electrodes. Herein, an efficient ETL material that enables low-temperature processing is presented. Tin dioxide (SnO₂) is modified by (sulfobetaine-*N,N*-dimethylamino)propyl naphthalene diimide (NDI-B) and used as an ETL. The NDI-B effectively reduces the interfacial nonradiative recombination between the ETL and perovskite and suppresses the ion migration by passivating oxygen-vacancy defects in SnO₂ and strongly interacting with halogen ions, respectively. Based on the NDI-B-blended SnO₂ ETL, a record PCE of 17.48% is achieved in the ITO-free flexible PSC fabricated at low temperature.

such as high light absorption coefficients, long electron-hole diffusion lengths, tunable bandgaps, and intrinsic mechanical flexibility.^[1–10] Numerous research groups have achieved power conversion efficiencies (PCEs) spanning from 3.81% to 26.08% in PSCs.^[11,12] To accelerate the commercialization of PSCs, current research endeavors have swiftly shifted the focus from rigid to flexible substrates, facilitating manufacturing by roll-to-roll printing methods. Various indium-tin-oxide (ITO)-free flexible transparent electrodes, such as conductive polymers, metal nanowires, and ultrathin metal film-based transparent electrodes have been developed.^[13–17] However, highly efficient PSCs have been realized only in planar n-i-p structures with metal-oxide electron transport layers (ETLs).^[12,18–20] Furthermore, these PSCs exhibit significant limitations that must be addressed for successful commercialization. Specifically, the processing temperature required is too high to be applied to flexible plastic substrates and transparent electrodes. This problem contrasts with the superior properties of PSCs, including their roll-to-roll manufacturability and potential flexible applications. In this context, developing functional ETL materials processable at low temperatures represents a significant challenge.

1. Introduction

Perovskite solar cells (PSCs) are considered at the forefront of next-generation photovoltaics owing to their superior properties

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Toward submicron inkjet-printed ZnO microdots with suppressed coffee-ring effect via controlled drying conditions for potential solar cell applications

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ABSTRACT

The high-resolution, inkjet-printed zinc oxide (ZnO) microdot arrays with suppressed coffee-ring effect was demonstrated by investigating the correlation between drying and solidification processes. The internal microfluidic behavior of an ejected droplet and the associated drying process is geometry-dependent, which can be controlled by the temperature and surface energy of the substrate. During evaporation, droplets in contact with a wettable surface exhibit a dominant outward convective flow, resulting in a pronounced coffee-ring effect. In contrast, for droplets with minimal contact area on a substrate with low surface energy, the surface energy gradient along the relatively long thermal conducting path length reinforces the Marangoni flow, which retards the pinning of the contact line, resulting in tiny microdots with suppressed coffee-ring effect. The controlled ZnO microdots exhibit a diameter of approximately 3 μm with a thickness of 50 nm, which is one of the smallest microstructures produced by the inkjet printing method. Additionally, the integration of the ZnO microdot arrays into organic solar cells aimed to alter the light path length, leading to enhanced internal absorption. The P3HT:PCBM, PTB7-Th:PCBM, and PM6:Y6 devices with ZnO microdot arrays exhibit higher power conversion efficiencies of 3.54%, 9.04%, and 15.61% compared to reference devices of 3.38%, 8.85%, and 15.25% respectively.

1. Introduction

Inkjet printing, a versatile and cost-effective method for precisely depositing soluble functional materials, offers significant potential for various applications across electronic and optoelectronic technologies [1–5]. One of the key advantages of inkjet printing is its ability to deposit materials with high precision, enabling intricate patterning without the need for highly sophisticated lithography processes [6–8]. Unlike traditional deposition techniques, such as physical vapor deposition or spin-coating, this maskless and noncontact approach simplifies the fabrication process and reduces production costs. Moreover, inkjet printing is highly adaptable and compatible with large-area continuous processes such as roll-to-roll printing, making this technology particularly attractive for a wide range of applications in both research and

industrial fields [9–12]. The scalability of this technology allows for the efficient production of electronic devices on flexible substrates, opening up new opportunities for the advancement of wearable electronics, flexible displays, and other innovative technologies [13–18].

In printed electronics, inkjet printing techniques are being actively explored beyond the use of conductive inks (e.g., metal nanowires/nanoparticles, conductive polymers, carbon nanotubes, or graphene), extending to semiconductor materials (e.g., polymers, small molecules, quantum dots, metal oxides) for solution processes [19–27]. Achieving reliable inkjet droplet formation is paramount for precise printing, necessitating consideration of rheological parameters associated with solvent properties, including vapor pressure, surface tension, and polarity [28–32]. In addition, recent advances have shed light on phenomena related to the drying process such as pinning and the coffee-ring

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RESEARCH ARTICLE

Effect of side chain modification on edge-on oriented dithienobenzodithiophene-based non-fullerene acceptors for organic solar cells

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Abstract

Two non-fullerene acceptors (NFAs), DTBBDT-ICN and DTBBDT-SEH, based on dithienobenzodithiophene (DTBBDT) and a 2-(3-oxo-2,3-dihydroinden-1-ylidene) malononitrile (IC) with different side chains of alkylthienyl and alkylthio-thienyl, respectively, were designed and used as electron acceptors in organic solar cells (OSCs). Both NFAs provide suitable energy level configurations that ensure efficient charge transfer with the donor polymer PBDB-T, as confirmed by significant photoluminescence reduction in the blend films. However, due to the high planarity together with strong π - π stacking interactions, the DTBBDT-ICN presented significant aggregation and phase separation in the blend films, leading to suboptimal charge generation. In addition, grazing incidence wide-angle x-ray scattering measurements revealed a predominance of edge-on molecular orientations, which are unfavorable for vertical charge transport. On the other hand, DTBBDT-SEH exhibited less pronounced molecular aggregation and edge-on orientation properties compared to DTBBDT-ICN, resulting in improved carrier mobility (μ_e of 3.86×10^{-6} compared to 7.59×10^{-7}) and mitigated recombination losses (1.19 kT/q compared to 1.21 kT/q) in OSC devices. The improved morphological features of PBDB-T:DTBBDT-SEH led to a high power conversion efficiency of 3.31%, which is three times higher than that of PBDB-T:DTBBDT-ICN-based devices (1.55%).

Da In Kim and Kyungsik Kim contributed equally to this work.

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Impact of Chemical Source Selection on Aqueous Spray-Deposited CZTSSe Solar Cells

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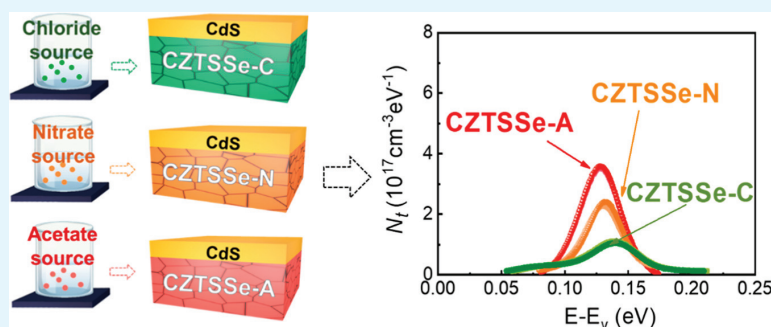
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ABSTRACT: The quest for a simple and cost-effective fabrication process to achieve high power conversion efficiency (PCE) in $\text{Cu}_2\text{ZnSn}(\text{S},\text{Se})_4$ (CZTSSe) solar cells has become increasingly important. Aqueous spray deposition is a promising method for the production of environmentally friendly and economically viable thin-film solar cells. In this study, we investigated the use of acetate, nitrate, and chloride sources in the aqueous spray deposition of CZTSSe. The effect of these chemical sources on the opto-electric and defect properties of the resulting thin films was thoroughly analyzed. Results showed that the acetate source resulted in high defect densities and a significant number of Cu_{Zn} antisites, while the use of nitrate and chloride sources improved solution stability and performance of the CZTSSe solar cells. The chloride-based solution was found to be the most stable, offering a unique opportunity for a reusable and cost-effective fabrication process. This study highlights the importance of chemical source selection in aqueous fabrication for the production of high-quality CZTSSe solar cells.

KEYWORDS: aqueous solution, acetate source, nitrate source, chloride source, defect properties, device performances, temperature-dependent $J-V$ measurements

1. INTRODUCTION

In recent years, there has been a growing interest in developing earth-abundant kesterite $\text{Cu}_2\text{ZnSn}(\text{S},\text{Se})_4$ (CZTSSe) solar cells due to their desirable properties of tuneable band gap (1.0–1.5 eV) and high light absorption coefficient ($>10^4 \text{ cm}^{-1}$).^{1–3} Various fabrication methods for CZTSSe have been developed, including both vacuum-based and nonvacuum-based approaches.^{4,5} Among these methods, solution-based approaches have shown promise in achieving high power conversion efficiencies (PCEs) by precisely controlling the elemental ratio and absorber thickness.^{6,7}

However, many of the solution-based approaches use toxic and flammable solvents, such as butylamine (BA), dimethyl sulfoxide (DMSO), ethanedithiol (ED), dimethylformamide (DMF), and ethanolamine (MEA), which require special fabrication conditions in nitrogen (N_2) or argon (Ar_2)-filled environments.^{8–10} To overcome this challenge, researchers are exploring alternative, eco-friendly aqueous solution approach

based on simple di-ionized water (DIW) and have achieved promising results with ambient air conditions.^{11,12}

The aqueous solution approach using DIW has the potential to overcome the challenges associated with traditional solvents, but it also presents new challenges such as the stability of the precursor solution and control of the elemental ratio. To further advance the field, this study investigates the effect of different starting materials on the aqueous precursor solution and the resulting CZTSSe device performances.

In this work, we proposed three different routes using acetate, nitrate, and chloride for aqueous solution-based CZTSSe device fabrication and achieved widely varied PCEs

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Achieving highly efficient kesterite solar cells using simultaneous surface Ge substitution and rear interface engineering strategies

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ABSTRACT

The progress in the device performance of kesterite-based thin-film solar cells (TFSCs) have been stagnant due to unstable rear contact properties and large open circuit voltage (V_{oc})-deficit characteristics. Several individual strategies have been developed to solve these issues. In this work, we investigate the simultaneous impact of Ge substitution and rear interface engineering with a MoO_x intermediate layer on Mo back contact. The simultaneous application of both strategies delivers a synergetic effect, improving microstructure, V_{oc} -deficit, band-tailing states, and device parameters and reducing Sn-related deep-level defects, resulting in enhanced power conversion efficiency (from 8.1 to 11.55%). This effective strategy offers new insight and a way to solve unstable rear contact properties and large V_{oc} -deficit, further improving the device performances of kesterite-based TFSCs.

1. Introduction

Kesterite-based $\text{Cu}_2\text{ZnSn}(\text{S,Se})_4$ (CZTSSe) semiconductor materials are evaluated as an attractive absorber candidate for next-generation thin-film solar cells (TFSCs) owing to their earth-abundant elements and superior optoelectronic properties [1–5]. Despite outstanding advantages, the currently reported highest power conversion efficiency (PCE) of a CZTSSe TFSC is only 14.9% [6], which is lower than that of the commercialized $\text{Cu}(\text{In,Ga})\text{Se}_2$ (CIGS) and CdTe TFSCs, which exhibit a PCE of over 22% [7,8]. The lower PCE of CZTSSe is mainly observed due to the relatively high open circuit voltage (V_{oc})-deficit and unstable back contact properties [9,10]. To minimize this large V_{oc} -deficit characteristic, various strategies have been successfully investigated, such as (i) rear contact interface engineering by introducing intermediate layers on rear contact such as metal [11–14] and metal oxide layers, [10,15–20] (ii) fine-tuning the conduction band offset using wider E_g materials, [21–23] and (iii) suppressing anti-site defect densities by introducing alkali [24–26] or partially alloying and/or doping cations in

kesterite [9,27,28].

When thick interfacial $\text{Mo}(\text{S,Se})_2$ layers are formed at high temperatures during the sulfo-selenization process, the instability of Sn ionic states could be accelerated, resulting in the formation of secondary phases and voids near rear back contact [29]. Thus, the introduction of thin metal and/or metal-oxide intermediate layer on the Mo back contact strategy can significantly suppress the unstable ionic Sn chemical reaction, formation of thick $\text{Mo}(\text{S,Se})_2$ interfacial layers, voids, the locally non-stoichiometric uniformity, unexpected defects or defect cluster, and secondary phases near the back contact region during the high-temperature annealing process [15,30]. Among several intermediate layers, MoO_x intermediate layer successfully demonstrated that it has minimal impact on the compositional ratios in kesterite while improving its microstructure [15,16,30,31].

Meanwhile, the anti-site defects (i.e., Cu_{Zn} and Zn_{Cu}) and related defect clusters are favorably formed during the annealing process at a relatively low temperature and in a short annealing time under a chalcogen vapor atmosphere [32,33]. In particular, the Sn-related defects

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RESEARCH ARTICLE

Vertical plane depth-resolved surface potential and carrier separation characteristics in flexible CZTSSe solar cells with over 12% efficiency

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Abstract

$\text{Cu}_2\text{ZnSn}(\text{S,Se})_4$ (CZTSSe) solar cells have resource distribution and economic advantages. The main cause of their low efficiency is carrier loss resulting from recombination of photo-generated electron and hole. To overcome this, it is important to understand their electron-hole behavior characteristics. To determine the carrier separation characteristics, we measured the surface potential and the local current in terms of the absorber depth. The elemental variation in the intragains (IGs) and at the grain boundaries (GBs) caused a band edge shift and bandgap (E_g) change. At the absorber surface and subsurface, an upward E_c and E_v band bending structure was observed at the GBs, and the carrier separation was improved. At the absorber center, both upward E_c and E_v and downward E_c -upward E_v band bending structures were observed at the GBs, and the carrier separation was degraded. To improve the carrier separation and suppress carrier recombination, an upward E_c and E_v band bending structure at the GBs is desirable.

KEYWORDS

carrier separation, CZTSSe, flexible solar cell, local current, surface potential

Dae-Ho Son, Ha Kyung Park, and Kee-Jeong Yang contributed equally to this study.

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Understanding of the Relationship between the Properties of Cu(In,Ga)Se₂ Solar Cells and the Structure of Ag Network Electrodes

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The relation between the structure of the silver network electrodes and the properties of Cu(In,Ga)Se₂ (CIGS) solar cells is systemically investigated. The Ag network electrode is deposited onto an Al:ZnO (AZO) thin film, employing a self-forming cracked template. Precise control over the cracked template's structure is achieved through careful adjustment of temperature and humidity. The Ag network electrodes with different coverage areas and network densities are systemically applied to the CIGS solar cells. It is revealed that predominant fill factor (FF) is influenced by the figure of merit of transparent conducting electrodes, rather than sheet resistance, particularly when the coverage area falls within the range of 1.3–5%. Furthermore, a higher network density corresponds to an enhanced FF when the coverage areas of the Ag networks are similar. When utilizing a thinner AZO film, CIGS solar cells with a surface area of 1.0609 cm² exhibit a notable performance improvement, with efficiency increasing from 10.48% to 11.63%. This enhancement is primarily attributed to the increase in FF from 45% to 65%. These findings underscore the considerable potential for reducing the thickness of the transparent conductive oxide (TCO) in CIGS modules with implications for practical applications in photovoltaic technology.

1. Introduction

In the field of solar energy, the promise of thin film technologies embodied by Cu(In,Ga)Se₂ (CIGS) and perovskite solar cells lies in their remarkable ability to reduce module thickness. This attribute not only results in lightweight configurations but also enables the creation of flexible solar modules. These inherent benefits have positioned thin-film solar cells as promising contenders for the next generation of solar technology, potentially replacing traditional Si solar cells. The fabrication process of thin-film solar modules typically involves the monolithic integration of subcells, a technique faced with two major challenges: resistive losses in transparent conductive oxides (TCO) and the occurrence of dead areas.^[1–4] The resistive losses are particularly problematic as the conductivity of TCO decreases with increased length, which restricts the width of individual subcells to about 4–5 mm to reduce these losses.^[4,5] Additionally, this limitation necessitates more frequent PIP2P3 scribing, leading to increased

dead areas and degradation of the thin film,^[1,4–6] which significantly contributes to the efficiency gap between solar modules and laboratory-scale cells.

Addressing these challenges, the application of a metal grid to the TCO as a front contact has been explored as an effective method to improve solar module performance by increasing the monolithic width.^[1,3,5–7] Research into the optimal structure of the metal grid has indicated that finger widths of 20–40 μm enhance the efficiency of solar cells.^[3–7] However, the relatively large width of the metal grid presents integration challenges in building structures and semi-transparent solar cells, particularly in terms of architectural design and aesthetics.^[8] Moreover, the reliance on expensive patterning techniques for strip lines, such as conventional lithography, has prompted investigations into simpler, cost-effective methods for applying metallic transparent electrodes.^[8–16]

The utilization of a self-formable cracked template represents a more straightforward and accessible approach compared with conventional lithography for manufacturing purposes. This method involves applying a solution to a substrate via spin coating, followed by a natural drying process that induces crack formation, serving as sacrificial templates.^[11,12,17–19] Such a technique facilitates the production of

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PAPER

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Reducing carrier recombination loss by suppressing Sn loss and defect formation via Ag doping in $\text{Cu}_2\text{ZnSn}(\text{S},\text{Se})_4$ solar cells†

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In this study, we analyzed the effect of the position of Ag in the stacked precursor structure of CZTSSe solar cells. Five precursor structures were designed by adding a 5-nm-thick Ag layer to soda-lime glass (SLG)/Mo/Zn/Cu/Sn at various positions, and CZTSSe devices were fabricated through a sulfo-selenization process. The SLG/Mo/Ag/Zn/Cu/Sn precursor structure device (C2) showed the best efficiency. This improvement is attributed to Ag promoting grain growth by forming a Cu–Sn alloy at a low temperature and suppressing the formation of defects and defect clusters. Conversely, the SLG/Mo/Zn/Ag/Cu/Sn precursor structure device (C3) hindered Cu–Zn interdiffusion, degrading the performance. C2 exhibited a small difference between the bandgap energy (E_g) and the photoluminescence, a high activation energy (E_A/E_g), and a long carrier lifetime, indicating reduced defect and carrier recombination loss. This study suggests that the location of Ag plays an important role in optimizing the CZTSSe efficiency. Additionally, a precursor containing Ag has been shown to suppress Sn loss during the sulfo-selenization process and improve device performance through liquid-assisted grain growth. This study shows that the location of Ag plays an important role in suppressing the carrier recombination loss of CZTSSe devices.

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Broader context

Renewable energy is regarded as highly important, and solar photovoltaic (PV) devices in particular are expected to play a significant role in reducing carbon dioxide emissions. To address the challenges posed by climate change in the current PV field, a wide range of technologies should be developed. For example, CuInGaSe_2 (CIGSe) solar cells require rare elements; thus, kesterite ($\text{Cu}_2\text{ZnSn}(\text{S},\text{Se})_4$, CZTSSe) solar cells have been developed as another type of thin-film solar cell to replace CIGSe. Several studies have been conducted to improve the efficiency of CZTSSe solar cells, and the highest efficiency was achieved through a solution process. For CZTSSe solar cells to enter the market, their efficiency must be enhanced through a vacuum process similar to that used for CIGSe solar cells. In addition to these improvements in efficiency, technology that can produce flexible and large-area modules with homogeneous properties is necessary for commercialization of CZTSSe solar cells.

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1. Introduction

$\text{Cu}_2\text{ZnSn}(\text{S},\text{Se})_4$ (CZTSSe) solar cells composed of earth-abundant materials have been reported to have an efficiency of more than 14% in recent research.¹ However, compared with the photovoltaic conversion efficiency (PCE) of 23.6% for $\text{Cu}(\text{In},\text{Ga})\text{Se}_2$ (CIGSe) solar cells,² the PCE of CZTSSe still needs to be improved. Compared with CIGSe, CZTSSe has a lower open-circuit voltage (V_{OC}) and current density (J_{SC}) characteristics. The V_{OC} of high-PCE CIGSe is greater than 0.7 V,^{2–4} whereas that of high-PCE CZTSSe is less than 0.6 V.^{1,5,6} The V_{OC}



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Cu-catalyzed aerobic oxidative dehydrogenation of tertiary indolines to indoles using azo/hydrazide redox†

Santanu Maiti, Jun Soo Kim and Jinho Kim *

Azo compounds are versatile in multifarious areas. They are crucial reagents in Mitsunobu reactions and dehydrogenations. However, the azo compounds or their corresponding hydrazides have never been investigated in the catalytic dehydrogenation of tertiary amines. Herein, we report a co-catalytic system using CuCl, 4-(dimethylamino)pyridine (DMAP), and 2-(4-nitrophenyl)hydrazine-1-carboxylate for the aerobic oxidative dehydrogenation of tertiary indolines to their corresponding indoles.

The dehydrogenation of saturated N-heterocycles to their corresponding N-heteroaromatic compounds is a fundamental and fascinating transformation in synthetic, materials, and medicinal chemistry.¹ The dehydrogenation of saturated N-heterocycles is conventionally conducted using stoichiometric amounts of oxidants such as DDQ,² metal oxides,³ and others,⁴ however, these methods are less attractive because of the toxicity and waste disposal associated with the oxidants. In recent years, transition metal-catalyzed acceptorless dehydrogenation has been regarded as a promising route for the synthesis of N-heteroaromatic compounds,⁵ because acceptorless dehydrogenation requires no stoichiometric oxidants and produces only molecular hydrogen as a byproduct.

Aerobic oxidative dehydrogenations provide another approach in terms of sustainability and green chemistry because molecular oxygen is abundant and readily accessible, and water is produced as the sole byproduct.⁶ Various catalytic systems have been developed to facilitate aerobic oxidative dehydrogenations of saturated N-heterocycles with one or more free N–H bonds.⁷ However, the aerobic dehydrogenations of N-substituted heterocycles such as tertiary indolines have been challenging (Scheme 1A).^{8–12} Recently, a couple of successful examples were reported. The Stahl group developed a [Ru(phd)₃](PF₆)₂-catalyzed aerobic dehydrogenation of

tertiary indolines to indoles,¹³ and Yang and co-worker reported that tertiary indolines efficiently underwent Mn-catalyzed aerobic dehydrogenation in the presence of AcOH as a solvent (Scheme 1B).¹⁴ Regardless of these advances, the development of more practical aerobic dehydrogenation methods for tertiary indoles, which are prevalent in bioactive compounds and pharmaceuticals, is still desirable. Herein, we describe a Cu-catalyzed aerobic oxidative dehydrogenation of tertiary indolines using ethyl 2-(4-nitrophenyl)hydrazine-1-carboxylate (**1**) as a co-catalyst (Scheme 1C).

Our group has developed various aerobic oxidative transformations using azo compounds. The azo compounds could be utilized as not only reagents for the synthesis of heterocycles¹⁵ but catalytic oxidants for dehydrogenations.¹⁶ Recently, we reported an aerobic oxidative dehydrogenation of secondary amines, such as tetrahydroquinolines using Mn(Pc)/**1** catalyst system.¹⁷ In this system, **1** was converted into ethyl 2-(4-nitrophenyl)diazene-1-carboxylate (**2**) by Mn(Pc) and O₂, and the generated **2** was reduced back to **1** by obtaining two electrons and two protons from the tetrahydroquinoline (one is from α -position of nitrogen and the other is from N–H) (Scheme 2A).¹⁸ These co-catalytic systems facilitate the aerobic oxidative dehydrogenation of tetrahydroquinolines to produce quinolines and water. Our persisting studies on aerobic oxidative dehydrogenation using **2/1** redox let us become interested in more challenging substrates such as tertiary indolines. Based on our mechanistic insight, we envisioned that the proton at the C3 position of indoline might be deprotonated by the generated hydrazide anion (**3**), if no acidic N–H proton was present, which would facilitate the dehydrogenation of more challenging tertiary indolines to indoles (Scheme 2B).

To begin, we carried out the dehydrogenation of 5-chloro-1-methylindoline (**4**) using stoichiometric amounts of **2**. The desired 5-chloro-1-methylindole (**5**) was produced in 90% yield with **1** as a byproduct (Scheme 3). Next, we investigated the feasibility of aerobic oxidative dehydrogenation of tertiary indolines using catalytic amounts of **1** (Table 1). Various catalysts, which can aerobically facilitate the **2/1** redox cycle,¹⁹ were screened (entries 1–4), and the CuCl/4-(dimethylamino)pyridine

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Article

Synergistic Antioxidant and Anti-Inflammatory Effects of Phenolic Acid-Conjugated Glutamine–Histidine–Glycine–Valine (QHGV) Peptides Derived from Oysters (*Crassostrea talienwhanensis*)

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Abstract: The glutamine–histidine–glycine–valine (QHGV), a peptide derived from oysters, exhibits antioxidant activity and is being actively researched as a potential pharmaceutical and functional cosmetic ingredient. In this study, we synthesized the QHGV peptide and explored the hitherto unknown anti-inflammatory effects of QHGV. The antioxidant property was also characterized by conjugating with various naturally derived phenolic acids, such as caffeic, gallic, ferulic, sinapinic, and vanillic acids. Conjugation with phenolic acids not only enhanced the antioxidant activity of QHGV but also diminished the lipopolysaccharide-induced generation of reactive oxygen species (ROS) in the murine macrophage cell line, RAW 264.7. The reduction in the levels of reactive oxygen species led to the reduced mRNA expression of inducible nitric oxide synthase (*iNOS*) and cyclooxygenase 2 (*Cox-2*), resulting in an anti-inflammatory effect through the inhibition of the phosphorylation of mitogen-activated protein kinase, including extracellular signal-activated protein kinase, c-Jun NH2-terminal kinase, and p38. Furthermore, the phenolic acid-conjugated peptides increased the mRNA and protein levels of collagen type I, indicative of a wrinkle-improvement effect. The phenolic acid conjugates of the peptide were not cytotoxic to human keratinocytes such as HaCaT cells. These results suggest that phenolic acid conjugation can enhance the potential of peptides as drug and cosmetic resources.

Keywords: antioxidant; anti-inflammatory; anti-wrinkle; natural peptide; cosmetic material; phenolic acids



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1. Introduction

The pursuit of beauty and healthy skin is a universal quest that impacts the self-expression and confidence of an individual and has cemented its significance in scientific research [1]. The identification of efficacious antiaging interventions is a fascinating and rapidly evolving area of study [2]. Among various antiaging agents, peptides have emerged as a novel class of cosmetic ingredients, with the industry seeking to maximally capitalize on their potential and multifunctionality [3]. These amino acid polymers have diverse biological functions, including the promotion of cell proliferation and anti-inflammatory, antioxidant, and anti-wrinkle benefits [4,5]. The pioneering synthesis of glutathione and the first cosmetic application of the copper-conjugated glycine–histidine–lysine (Cu-GHK) peptide in the late 1980s are landmarks in the innovative trajectory of peptide utilization [6,7]. Exhibiting high efficacy at low concentrations and high stability characteristics, peptides hold great promise owing to their potent physiological activities [8].

Mn-Catalyzed Aerobic Oxidative α -Cyanation of Tertiary Amines Using Azo/Hydrazide Redox

Su Been Kim,[▽] Gayeong Park,[▽] Eun Sun Park, Santanu Maiti, and Jinho Kim*



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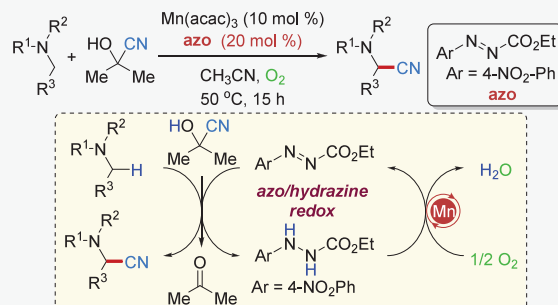


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Supporting Information

ABSTRACT: Azo compounds such as diethyl azodicarboxylate have been used in oxidative coupling reactions to generate iminium ions from tertiary amines. However, the requirement of stoichiometric amounts of azo compounds limits their large-scale applications. Herein, we present an aerobic oxidative α -cyanation of tertiary amines using catalytic amounts of an azo compound or hydrazine. The developed protocol provides a practical and ecofriendly route for α -cyanated tertiary amines, using molecular oxygen as the terminal oxidant.



α -Amino nitriles have been used as versatile intermediates in organic synthesis.¹ They can be converted into other important functional groups such as α -amino acids or diamines through hydrolysis or reduction of the nitrile, respectively.² They have been utilized as stable precursors to iminium ions that were subsequently trapped with various nucleophiles and as building blocks for various biologically active N-heterocycles due to their bifunctional reactivities.³

The representative synthetic route for α -amino nitriles is the three-component reaction of aldehydes, amines, and cyanides, which was reported in 1850 by Strecker.⁴ Since Strecker's first report, a variety of synthetic procedures has been extensively developed.⁵

Recently, oxidative coupling reactions between tertiary amines and cyanide sources have emerged as alternative approaches for α -amino nitriles. In these oxidative coupling reactions, it was generally proposed that the desired α -amino nitriles were produced by the nucleophilic additions of cyanide sources to the in situ-generated iminium ion intermediates.⁶ To generate the key intermediate, iminium ion, a variety of catalytic oxidative systems,⁷ photoredox systems,⁸ and electrochemical systems⁹ were developed. Strategies using only stoichiometric oxidants such as PhI(OAc)₂, DDQ, and oxoammonium salt were also reported.¹⁰

Azo compounds such as diethyl azodicarboxylate (DEAD) are useful in organic synthesis.¹¹ These azo compounds are key reagents in the Mitsunobu reaction¹² and useful electrophiles in other reactions.¹³ In 2008, Li and co-workers reported that diethyl azodicarboxylate (DEAD) could act as the oxidant to generate iminium ions from tertiary amines.¹⁴ After their pioneering works on DEAD-mediated oxidative coupling reactions of tertiary amines with terminal alkynes,¹⁵ sub-

sequent studies have shown that a wide range of nucleophiles could be used in these reactions (Figure 1A).¹⁶ However, the use of stoichiometric amounts of DEAD is undesirable for a large-scale process, and the generation of diethyl hydrazodicarboxylate as a byproduct complicates the purification process. Therefore, the development of practical oxidative coupling reactions using catalytic amounts of stable azo compounds is highly desirable.

Our group has focused on the development of aerobic oxidative transformations using hydrazines.¹⁷ In our recent study, we disclosed the aerobic oxidative dehydrogenations of secondary amines and tertiary amines employing a catalytic amount of 2-(4-nitrophenyl)hydrazine-1-carboxylate (**1**).¹⁸ We proposed that **1** was converted into ethyl 2-(4-nitrophenyl)-azodicarboxylate (**2**) by transition metal-catalyzed aerobic oxidation, and the generated **2** was reduced back to **1**, facilitating the dehydrogenation of amines. This co-catalytic system allows the utilization of molecular oxygen as the terminal oxidant. Building upon our mechanistic insight, we envisioned that our developed catalytic 1/2 redox system could provide a practical and alternative route for DEAD-mediated oxidative coupling reactions (Figure 1B). Herein, we report the Mn(acac)₃-catalyzed aerobic oxidative α -cyanation of tertiary amines using catalytic 1/2 redox.

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Sequential Energy and Electron Transfer in Metal–Organic Frameworks

Wooseong Jo,[‡] Hyun Seok Lee,[‡] Tra Phuong Trinh, Gajendra Gupta, Miyeon Kim, Ga Young Kim, Jinho Kim, Chul Hoon Kim,^{*} and Chang Yeon Lee^{*}



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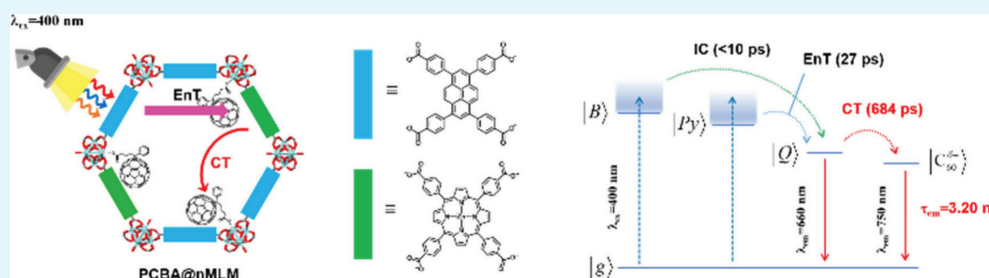
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ABSTRACT: This study presents the design and characterization of a triad metal–organic framework (MOF) system composed of pyrene, porphyrin, and phenyl- C_{61} -butyric acid (PCBA) for efficient energy and electron transfer processes mimicking natural photosynthesis. The triad MOF, synthesized through a mixed-ligand approach followed by postsynthetic modification, demonstrates sequential energy transfer from pyrene to porphyrin, followed by electron transfer to the PCBA acceptor. Time-resolved photoluminescence (TRPL) spectroscopy was employed to investigate the dynamics of energy and charge transfer, revealing fast interligand energy transfer and subsequent charge separation in the MOF structure. The PCBA-functionalized MOF (PCBA@nMLM) exhibited a significantly enhanced photocatalytic performance compared to the nonfunctionalized counterpart, particularly in the selective aerobic oxidation of sulfides to sulfoxides under visible light irradiation. The enhanced photocatalytic activity is attributed to the prolonged charge separation facilitated by the PCBA moieties, as confirmed by electrochemical impedance spectroscopy (EIS) and transient photocurrent measurements. This work highlights the potential of MOF-based systems in artificial photosynthesis and other photocatalytic applications by effectively harnessing solar energy through optimized energy and charge transfer mechanisms.

KEYWORDS: energy transfer, metal–organic frameworks (MOFs), electron transfer, photocatalysis, light-harvesting

1. INTRODUCTION

Photosynthesis involves the process by which plants and some organisms use solar energy to convert carbon dioxide and water to organic compounds and oxygen. This process relies on two crucial steps, known as sequential energy and electron transfer. The first energy transfer step in photosynthesis is collecting and transferring light energy.¹ During this step, the photosynthetic system gathers energy from light-absorbing tissues. The photosynthetic system responsible for this action consists of two key components: the photosystems (photosystem I and photosystem II) and pigments (such as chlorophyll and carotenoids). The photosystems absorb light energy and excite electrons, which are then transferred to the pigments. This energy transfer is crucial for the efficiency and stability of the photosynthetic process. Notably, the photosystems play different roles based on the wavelength of light, enabling the efficient utilization of a wide range of optical light in photosynthesis. The ordered pigment arrays are essential for

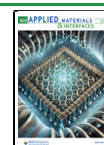
optimizing light absorption and facilitating efficient energy transfer.^{2,3} The second step, electron transfer, occurs once the light energy is transferred to the pigments.⁴ The excited electrons move through an electron transfer chain from the pigments to the electron acceptors during this step. This process involves the transfer of energy and triggers chemical reduction reactions. The chemical reduction reactions generated during electron transfer subsequently induce various chemical reactions that synthesize organic compounds from carbon dioxide and water. Additionally, the electron transfer process allows the separation and distribution of charges

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ARTICLE

1,1-Diethoxyethane as an interfacial-stabilizing solvent for lithium–sulfur batteries

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Abstract

Lithium–sulfur batteries (LSBs) have gained remarkable attention over the past several years, however, their inherent limitations, such as the poor interfacial stability of their Li anodes and the severe dissolution of polysulfide, are regarded as bottlenecks that prevent these batteries from entering commercial markets. In this study, a 1,1-diethoxyethane (DEE), which has been functionalized with acetal groups, is proposed as an electrolyte cosolvent to complement the limitations of the electrode materials of LSBs. In Li/Li symmetric cells, the DEE cosolvent exhibited stable cycling behavior, whereas the cell with the baseline electrolyte exhibited rapidly increasing polarization, even after 650 h. In LSB cells, the electrolyte that contains 50% DEE exhibits the highest cycling retention (65.0%) compared to the baseline electrolyte (49.8%) because it effectively protects the interface of the Li anode, but also suppresses the dissolution of polysulfide species.

KEYWORDS

1,1-diethoxyethane (DEE), electrolyte, lithium–sulfur batteries, polysulfide dissolution, solid-electrolyte interphases

INTRODUCTION

The rapid growth in the worldwide market for electricity generated from renewable energy sources has been accompanied by an exponential increase in the demand for rechargeable batteries as the primary energy source for electric vehicles. Unfortunately, the energy density of current state-of-the-art lithium-ion batteries of approximately 250 W h kg^{−1} is insufficient to satisfy the specifications of large-scale devices.^{1–5} This has prompted numerous studies over the past several years to increase the energy density of conventional rechargeable batteries.^{6–12} In this respect, lithium–sulfur batteries (LSBs) have gained considerable attention as advanced-generation batteries because they can realize energy density that is almost 10 times higher than that of conventional rechargeable batteries, owing to the high specific capacity of the lithium anodes and sulfur cathode materials of which LSBs are composed.^{13–15}

However, LSBs have critical limitations in terms of realizing stable cycling performance for prolonged periods of time. Although sulfur cathode materials can theoretically provide a specific capacity that is almost 10 times higher

(1675 mA h g^{−1}) than that of the conventional cathodes used in lithium-ion batteries, a much lower specific capacity is observed in practical cells. This is because of the low electrical conductivity of sulfur (5×10^{-30} S cm^{−1}), which hinders the intensive utilization of sulfur in electrochemical processes.^{16,17} In addition, the polysulfide (PS) intermediate formed during the electrochemical reaction in a cell is considered a critical bottleneck that adversely impacts the cycling retention of LSBs.^{18–20} In this regard, it should be noted that because these PS species are highly soluble in the electrolyte, they significantly degrade the cycling retention of LSBs. The degradation is caused by a shuttle reaction resulting from freely migrating PS species between the cathode and anode, which ultimately leads to the loss of active cathode materials upon cycling.^{21,22} It should be emphasized that the low electrical conductivity of active S cathode materials and the shuttle reaction of PS are regarded as the most challenging obstacles that need to be overcome to improve the electrochemical behavior of LSBs.

Attempts to resolve these issues most frequently involve the use of sulfur/carbon composites consisting of a mesoporous carbon host infiltrated with sulfur.



ORIGINAL ARTICLE

Regulating interfacial stability of SiO_x anode with fluoride-abundant solid–electrolyte interphase by fluorine-functionalized additive

Subin Lee, Taeun Yim* 

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Abstract Silicon oxide (SiO_x) has received remarkable attention as a next-generation battery material; however, the sudden decrease in the cycling retention constitutes a significant challenge in facilitating its application. Tris(2,2,2-trifluoroethyl) phosphite (TTFP), which can control parasitic reactions such as the pulverization of SiO_x anode materials and electrolyte decomposition, has been proposed to improve the lifespan of the cell. The electrochemical reduction of TTFP results in solid–electrolyte interphase (SEI) layers that are mainly composed of LiF , which occur at a higher potential than the working potential of the SiO_x anode and carbonate-based solvents. The electrolyte with TTFP exhibited a substantial improvement in cycling retention after 100 cycles, whereas the standard electrolyte showed acutely decreased retention. The thickness of the SiO_x anode with TTFP also changed only slightly without any considerable delamination spots, whereas the SiO_x anode without TTFP was prominently deformed by an enormous volume expansion with several internal cracks. The cycled SiO_x anode with TTFP exhibited less increase in resistance after cycling than that in the absence of TTFP, in addition to fewer decomposition adducts in corresponding X-ray photoelectron spectroscopy (XPS) analyses between the cycled SiO_x anodes. These

results demonstrate that TTFP formed SEI layers at the SiO_x interface, which substantially reduced the pulverization of the SiO_x anode materials; in addition, electrolyte decomposition at the interface decreased, which led to improved cycling retention.

Keywords Lithium-ion batteries (LIBs); Silicon oxide anode; Electrolyte; Additive; Solid–electrolyte interphases (SEI); Tris(2,2,2-trifluoroethyl) phosphite

1 Introduction

Owing to the increase in interest in eco-friendly devices in recent years, the demand for lithium-ion batteries (LIBs) has exponentially surged. Ever since LIBs were first employed in small-scale devices in the 1990s, they have been applied in electric vehicles and energy storage systems [1–7]. Accordingly, there is a current demand for advanced electrode materials that can significantly increase the energy density of LIBs because they tend to increase in proportion to the specific capacity of the electrode material [8–12]. Numerous attempts have been made to develop electrode materials that satisfy these requirements.

Silicon oxide (SiO_x) has recently received significant interest as an anode capable of achieving a high energy density [13–15]. In contrast to conventional graphite anode materials, SiO_x anode materials possess more than 10 times higher specific capacity than graphite anode materials based on an alloy reaction between the SiO_x anode material and Li^+ . However, SiO_x anode materials present an obvious limitation compared to conventional graphite anode materials in terms of low cycling retention, which originate from the poor reversibility of the lithiation/delithiation reactions. SiO_x anode materials can be converted to Li-Si

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Spartina anglica-Derived Carbon-Coated PE Separator for Physically Restraining Polysulfide Migration in Lithium-Sulfur Batteries

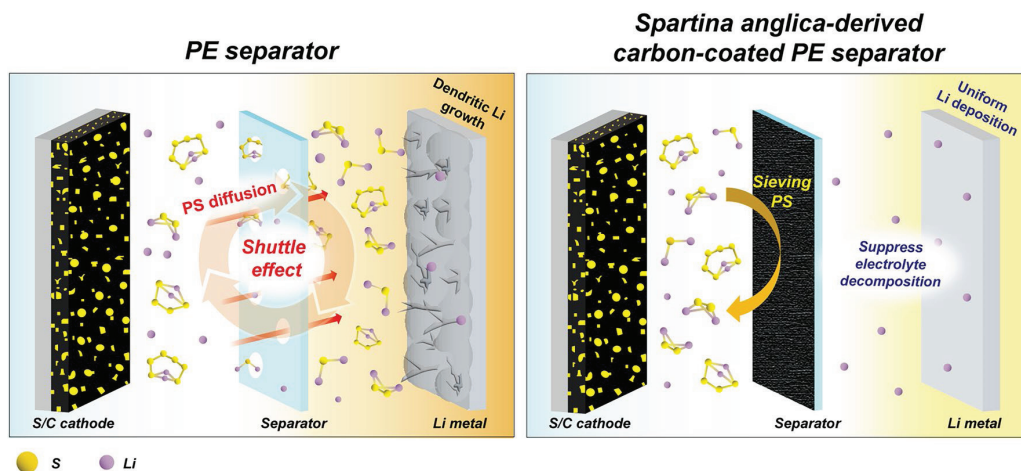
Ye Jin Jeon^{1,2} · Yuna Ha² · Jang Kyun Kim^{2,3} · Youn-Jung Kim^{2,3} · Taeun Yim^{1,2}

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Abstract

Lithium–sulfur batteries (LSBs) have received substantial interest because their theoretical energy density is considerably higher than that of conventional lithium-ion batteries. However, the difficulty in confining the soluble intermediate polysulfide (PS) species of LSBs hinders the prolonged cycling of the cell. In this study, a *Spartina anglica*-derived carbon-coated polyethylene (PE) separator (SC-coated PE separator), which can confine the PS species on the cathode side, is developed to improve the cycling retention of LSBs. *Spartina anglica*, which is considered a predominant marine waste, is converted to task-specific carbon materials via size-controlled milling and carbonization, and is embedded on a PE separator through a simple casting process. The SC-coated PE separator improves the electrolyte affinity, which is characterized by measuring the contact angle, electrolyte uptake, and transference number of Li⁺; consequently, the migration of Li⁺ is undisturbed in the cell, even if an additional layer is formed on the PE separator. Based on the electrochemical performance, the SC-coated PE separator exhibits a higher initial specific capacity than the PE separator, in addition to a remarkably increased cycling retention (65.3% vs. 27.7%) after 100 cycles. The SC-coated PE separator effectively inhibits the PS species through physical absorption, thereby increasing the utilization of the active sulfur species. Moreover, the SC-coated PE separator presents a relatively stable interfacial morphology of the cycled Li anode, revealing that the comprehensive interfacial stability of the Li–S cell is enhanced by effectively confining the PS species in the cell.

Graphical Abstract



Keywords Lithium-sulfur batteries · *Spartina anglica*-derived carbon · Separator · Confining polysulfide · Retention

Extended author information available on the last page of the article

Poly(acrylic acid) Grafted with a Boronic Ester and Dopamine as a Self-Healable and Highly Adhesive Aqueous Binder for Si Anodes

Yumi Kang, Sangwook Kim, Jonghyeok Han, Taeun Yim, and Tae-Hyun Kim*

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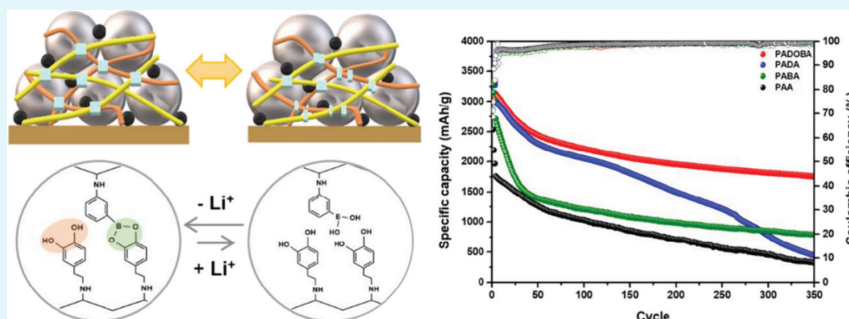
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Supporting Information



ABSTRACT: An aqueous cross-linked poly(acrylic acid) (PAA) polymer binder grafted with a boronic ester and dopamine, xPAA-B-DA, was developed herein for Si anodes. The reversible boronic ester bonds contribute to the self-healing characteristics of the binder, and dopamine improves its adhesive properties. A Si anode was fabricated by using xPAA-B-DA (Si@xPAA-B-DA), effectively inhibiting the volume expansion of Si while ensuring the structural integrity of the Si electrode. Unlike other studies employing self-healing boronic esters as a binder via boronic acid–diol cross-linking, this study adopts a novel approach by combining the cross-linking between boronic acid and the diol functionality in dopamine (involving self-healing properties) with intentionally retained dopamine to further increase the binder's adhesive performance. Accordingly, Si@xPAA-B-DA exhibits better adhesion and electrochemical performance than Si@xPAA-B (only containing cross-links between a boronic ester and a limited amount of dopamine) due to the synergy between the self-healing properties of boronic esters and the adhesive characteristics of dopamine. At a 0.5 C current density, the initial Coulombic efficiency is 74.9%; furthermore, even after 350 cycles, the capacity remains high at 1755.4 mAh g⁻¹, yielding a 53.7% preservation of capacity. Moreover, after 200 cycles at a higher current density of 1 C, Si@xPAA-B-DA maintains 63.0% of its capacity, or 1674.3 mAh g⁻¹. After 100 cycles, Si@xPAA-B-DA retains 70.8% of its capacity, which is notable even for a Si size of 350 nm.

KEYWORDS: polymer binder, Si anode, dynamic boronic ester bond, 3D network, self-healing property

1. INTRODUCTION

Extensive research efforts are presently in progress to expand the utilization of lithium-ion batteries (LIBs) from compact devices to larger-scale applications such as electric vehicles (EVs) and energy storage systems (ESSs). Consequently, there is an increasing demand for LIBs with higher energy densities. Developing such devices necessitates employing high-performance silicon (Si)-based materials with a notably high theoretical capacity as active materials for anodes.

The anode is one of the most important components of an LIB in determining the overall battery capacity. Graphite is currently widely used as an active anode material; however, despite its excellent cyclability, it is less suitable for large-scale energy devices due to its low theoretical capacity (372 mAh g⁻¹). In contrast, Si not only has a high capacity of 4200 mAh g⁻¹ but is also abundant and highly stable due to its low operating potential, making it highly suitable for high-capacity

ESSs. Given its potential advantages, Si has recently gained interest as a potential active material for LIB anodes.^{1–4}

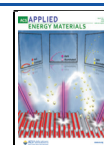
Nevertheless, the large volume change of up to 400% during the charge process, in which Si alloys with Li ions, followed by irreversible volume expansion during the discharge process presents a challenge for using Si as an active material. This phenomenon makes it difficult to restore the Si structure to its initial condition, thus resulting in cracks on and within the anode and causing the entire electrode structure to collapse.

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Simultaneous Realization of Multilayer Interphases on a Ni-Rich NCM Cathode and a SiO_x Anode by the Combination of Vinylene Carbonate with Lithium Difluoro(oxalato)borate

Ha Neul Kim, Soon Young Kim, Jungyoung Ahn, and Taeun Yim*

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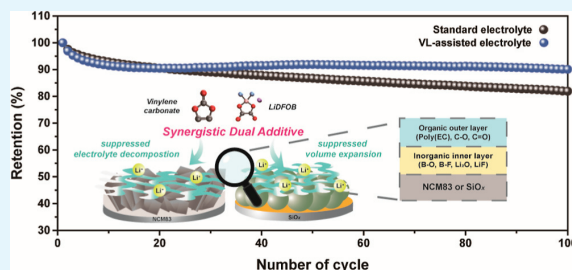
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Supporting Information

ABSTRACT: Ni-rich NCM and SiO_x electrode materials have garnered the most attention for advanced lithium-ion batteries (LIBs); however, severe parasitic reactions occurring at their interfaces are critical bottlenecks in their widespread application. In this study, an effective additive combination (VL) composed of vinylene carbonate (VC) and lithium difluoro(oxalato)borate (LiDFOB) is proposed for both Ni-rich NCM and SiO_x electrode materials. The LiDFOB additive individually delivers inorganic-rich cathode–electrolyte interphase (CEI) and solid–electrolyte interphase (SEI) layers in anodic and cathodic polarizations before the VC additive. Subsequently, the VC additive is capable of the formation of additional CEI and SEI layers composed of relatively organic-rich components through an electrochemical reaction; thus, inorganic–organic hybridized CEI and SEI layers are simultaneously formed at the Ni-rich NCM and SiO_x electrodes. Accordingly, the VL-assisted electrolyte exhibits remarkably prolonged cycling retention for the Ni-rich NCM cathode (86.5%) and SiO_x anode (72.7%), whereas the standard electrolyte shows a substantial decrease in cycling retention for the Ni-rich NCM cathode (59.2%) and SiO_x anode (18.1%). Further systematic analyses prove that VL-assisted electrolytes form effective interphases for Ni-rich NCM and SiO_x electrodes simultaneously, thereby leading to stable and prolonged cycling behaviors of LIBs that offer high energy densities.

KEYWORDS: Ni-rich NCM cathode, SiO_x anode, vinylene carbonate, lithium difluoro(oxalato)borate, solid–electrolyte interphase, cathode–electrolyte interphase



1. INTRODUCTION

Recently, there has been an increased interest in electric vehicles (EVs), which use electrical energy stored in Li-ion batteries (LIBs) to replace internal combustion engines that produce energy from fossil fuels.^{1–3} The performance of LIBs has garnered rising interest in recent years, given that it directly determines the driving range of EVs. Subsequently, high-energy-density, long-lifespan, and lightweight LIBs have become pivotal for electric vehicles. Among these, increasing the energy density of LIBs is the paramount issue in the energy society.^{4,5}

An effective approach to increase the energy density is the use of high-capacity electrode materials for LIBs. Currently, the theoretical specific capacity of conventional graphite anode materials is 372 mA h g^{−1}, with the Li⁺ intercalation reaction occurring at 0.1 V (vs Li/Li⁺).^{6,7} Despite the commercialization of graphite anodes, new anode materials are being actively studied owing to the low energy density of conventional LIBs. Recently, the research and development of Si-based anode materials has surged because of their specific capacity, which is almost ten times greater than that of conventional graphite, via alloy reactions with Li⁺.^{8–10} Nevertheless, such alloy reactions

always pulverize Si-based anode materials through rapid volume expansion and contraction upon cycling.^{11–13} Note that once Si-based anode materials are pulverized, the released dead Si-based anode material results in a significant decrease in the specific capacity. Simultaneously, electrolyte decomposition is accelerated at the interface of the Si-based anode (from pulverization), causing a rapid decline in the retention owing to an increase in resistance.^{14,15}

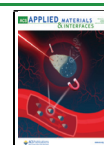
The limitations of Si-based negative electrode materials have been the subject of numerous studies. To alleviate the mechanical stress due to alloy reactions, there has been active research on ways to alter Si-based anode materials. There have been various attempts to (1) reduce the mechanical stress caused by volume change by downsizing the particle size of Si-

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journal homepage: www.elsevier.com/locate/jelechemPreserving the structural stability of $\text{LiNi}_{0.92}\text{Co}_{0.04}\text{Mn}_{0.04}\text{O}_2$ cathode by Ti^{4+} -ion doping for lithium-ion batteriesJun Won Heo^{a,b}, Giseung Lee^{a,b}, Byong-June Lee^c, Jeonghan Kim^{c,*}, Taeun Yim^{a,b,*}^a Advanced Batteries Laboratory, Department of Chemistry, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea^b Research Institute of Basic Sciences, College of Natural Science, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea^c Cathode Material R&D Group, POSCO FUTURE M, 31, Sojeongsandan 4-ro, Sojeong-myeon, Sejong-si 30002, Republic of Korea

ARTICLE INFO

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Titanium
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ABSTRACT

Given the exponential rising demand for high-performance lithium-ion batteries (LIBs), Ni-rich layered $\text{LiNi}_x\text{Co}_y\text{Mn}_{1-x-y}\text{O}_2$ (LN) cathode materials have gained significant attention. Nevertheless, poor structural stability of LN cathode materials is a critical hurdle to their use in LIBs. In this regard, Ti-doped LN cathode materials are designed to enrich the structural reliability of LN cathode materials, and their underlying behaviors are elucidated. Once Ti is replaced in the layered sites of the LN cathode materials, it strongly binds to lattice oxygen owing to its outstanding binding affinity. Therefore, Ti-doped Ni-rich LN cathode materials exhibit increased thermal stability and mechanical rigidity compared with bare LN cathode materials. In terms of cycling behavior, the Ti-doped Ni-rich LN cathode materials deliver remarkably improved cycling retention; the 2.0% Ti-doped Ni-rich LN cathode material yields an 88.0% retention, while the bare Ni-rich LN cathode material yields a 52.2% retention after 100 cycles. Further analyses of the recovered Ni-rich LN cathodes indicate that structural deformations, such as microcracks and phase transitions, are evidently suppressed in the Ti-doped LN cathode, and additional parasitic reactions, such as electrolyte decomposition, are also inhibited after cycling. This means that incorporating Ti in Ni-rich LN cathode materials not only suppresses structural deformation but also simultaneously increases the interfacial stability upon cycling, thus leading to a comprehensively improved cycling behavior.

1. Introduction

Following the worldwide consensus on the reduction of carbon dioxide emissions, numerous efforts are underway to transition away from energy systems that rely on fossil fuels. Lithium-ion batteries (LIBs) have received extensive notice as encouraging energy storage and conversion systems [1–6]. LIBs have been used in small-scale devices since their successful commercialization in the early 1990s. As the market for medium- and large-scale LIBs has experienced significant growth in recent years, securing a higher energy density for LIBs has become a prominent issue for the energy society. Note that the energy density of LIBs is mainly affected by the electrode materials used; therefore, advanced electrode materials offering high-energy densities have been intensively investigated [7–10].

Conventional LIBs employ LiCoO_2 as the main cathode material and exhibit an average discharge voltage of 3.8 V with a specific capacity of

150 mA h g^{−1} [11–13]. Although they have afforded stable cycling behavior, especially in small-scale LIBs, numerous efforts have been expended to identify advanced cathode materials for large-scale LIBs. Because the energy density is in proportion to the working potential of the cathode materials, a considerable amount of research has been conducted to develop advanced cathode materials with higher electrochemical potentials, such as Li-rich layered oxides [14,15] and spinel-type oxides, such as $\text{LiNi}_{0.5}\text{Mn}_{1.5}\text{O}_4$ [16–18]. Furthermore, the energy density is proportional to the specific capacity; therefore, cathode materials that can offer high-specific capacities have also been intensively developed. In particular, many studies on layered $\text{LiNi}_x\text{Co}_y\text{Mn}_{1-x-y}\text{O}_2$ (LN) cathode materials, derived by replacing Co with Ni and Mn in LiCoO_2 cathode materials, have exponentially progressed over the past several years [19–22]. Note that the Ni element occupying the layered site reacts electrochemically at a lower potential than the Co species; therefore, the capacity of LN cathode materials can be elevated by

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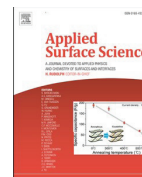
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Full Length Article

Boron- and calcium-interspersed cathode-electrolyte interphases on Ni-rich layered oxide cathodes for advanced performance lithium-ion batteries

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ABSTRACT

Although Ni-rich layered oxides (Ni-rich NCM) have become the preferred cathode materials in the electric vehicle market, their unsustainable cycling behavior arising from the extremely high reactivity at the interface has become a formidable challenge. To alleviate this problem, we propose modifying the cathode-electrolyte interphase (CEI) of Ni-rich L-NCM cathodes by incorporating B/Ca functional groups. Dual-functionalized CEI layers are prepared by heating boric acid (H_3BO_3) and calcium oxide (CaO) in a one-step process. Islands of B- and Ca-functionalized CEI layer are observed at the Ni-rich L-NCM cathode, and the thickness is controlled to approximately 2.4 nm. The B-functional group not only lowers the concentration of residual lithium but also compensates for the longer Li^+ migration distance by providing a desirable pathway based on ion-hopping sites. The Ca functional groups increase the mechanical rigidity of the Ni-rich L-NCM cathode, which inhibits the appearance of microcracks. In terms of its cycling behavior, the capacity retention of the cell with the B/Ca-incorporated Ni-rich L-NCM cathode improves markedly (72.4%), whereas the bare Ni-rich L-NCM material only retains 44.3% of its capacity after 100 cycles.

1. Introduction

The increasing commercial availability of lithium-ion batteries (LIBs) has shifted the market from small to large applications, and the required characteristics of LIBs have continued to diversify [1–5]. In particular, LIBs have received substantial attention as the favored power supply for electric vehicles to satisfy the ever-increasing demand for longer driving distances. Accordingly, numerous efforts have been conducted to find advanced electrodes capable of offering greater specific capacity than state-of-the-art electrode materials, considering the such requirements of LIBs [6–10]. Among the many candidates of cathodes, Ni-rich L-NCM cathode materials ($\text{LiNi}_x\text{Co}_y\text{Mn}_{(1-x-y)}\text{O}_2$, $x \geq 0.6$) have gained considerable attention, because their electrochemical performance can be adjusted by varying the Ni, Co, and Mn content of the layered structure [11–15]. The Mn component is known to contribute to increasing the structural reliability of L-NCM cathode materials as it can strongly bind with lattice oxygen, which is essential to suppress the release of oxygen during cycling [16–18]. In contrast, Ni and Co directly affect the reversible electrochemical reactions, which play a crucial role in determining the capacity of L-NCM cathode

materials [19–21]. In particular, the capacity of L-NCM cathode materials is highly dependent on the relative ratio of Ni and Co components. Because Ni^{2+} has a lower oxidation–reduction potential than Co^{3+} in this structure, the specific capacity can be improved in relative terms by ensuring that the layered structure is mainly occupied by Ni instead of Co [22–24]. This implies that the capacity of the L-NCM cathode materials would be controlled via changing the elements of the layered sites by adjusting the Ni content. This prompted the development of Ni-rich L-NCM cathode materials such as Ni 60% [25,26], Ni 70% [27,28], and Ni 80% [29,30] as well as more recent attempts to fabricate L-NCM cathode materials with Ni content of 90% or more [31–33].

Nonetheless, their lifespan rapidly deteriorates because of their poor interfacial stability. When these cathodes are charged (usually to 4.3 V (vs. Li/Li^+)), many extremely unstable Ni^{4+} species, which tend to be easily reduced, mainly appear at the L-NCM cathode [34]. It means that the constant contact between the L-NCM cathode and the electron-rich electrolyte during electrochemical cycling facilitates electrolyte decomposition because the Ni^{4+} species willingly accepts electrons from the electrolyte [35,36]. Once the electrolyte starts decomposing, the additional layers formed as a result thereof hinder Li^+ migration in the

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A sacrificial separator facilitating *in situ* creation of a durable CEI layer and tailoring lithium dendrites for practical lithium metal batteries†

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A Li metal anode and NCM811 cathode are used to realize practical Li metal batteries (LMBs) with a high energy density. However, during repeated charging–discharging processes, the concurrent occurrence of two adverse effects, *i.e.*, uncontrollable Li dendrite formation and the structural collapse of the Ni-rich cathode, results in severe degradation of the electrochemical performance of LMBs. To tackle these challenges, we propose an interface-targeting separator, which is prepared by anchoring tributyl borate (TBB) onto a polyethylene (PE) separator. The TBB–PE separator can facilitate the robust formation of a cathode–electrolyte interface (CEI) while suppressing the uncontrollable formation and growth of Li dendrites. The TBB–PE separator can tightly hold the anions of the dissociated Li salts, thereby facilitating the transport of Li⁺ and ultimately suppressing the formation and growth of dendritic Li. Concurrently, the TBB–PE separator forms a robust and uniform boron-rich CEI layer, thereby preventing the occurrence of electrolyte decomposition at the NCM811 cathode. Therefore, these distinctive characteristics of the TBB–PE separator allow enhanced cycling performance in the resulting LMBs assembled with Li metal and NCM811 in a carbonate electrolyte.

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1. Introduction

Lithium-metal batteries (LMBs) coupled with NCM811 cathodes are considered a standard combination for achieving high-energy-density LMBs.^{1–3} Theoretically, the energy density of LMBs assembled with Li metal and NCM811 is $\sim 500 \text{ W h kg}^{-1}$, which is almost two times higher than that of traditional lithium-ion batteries ($\sim 250 \text{ W h kg}^{-1}$) composed of commercial graphite and lithium cobalt oxide electrodes.^{4–8} However, the commercialization of LMBs comprising NCM811 remains challenging owing to the severe degradation of the electrochemical performance caused by the concurrent occurrence of two adverse effects: uncontrollable dendritic Li growth and the morphological irregularity of NCM811.^{9–13} Therefore, the

undesirable interfacial behaviors of the Li metal and NCM811 have been considered as the most critical limitations for developing practical high-energy-density LMBs.

Thus far, various strategies for effectively controlling the undesired parasitic reactions that occur at the Li metal and NCM811 have been proposed as independent approaches to separately address the challenges of Li metal and NCM811 materials. Numerous attempts, including the utilization of functional additives, protective artificial layers, separator modifications, and three-dimensional (3D) frameworks, have been made to adapt the surface structure of Li metal anodes.^{14–17} For NCM811, doping the layered structure and controlling the grain boundary of NCM811 as a single-crystal structure have been extensively investigated, with a focus on increasing the structural stability of NCM811 materials.^{18,19} Functional electrolyte additives have also attracted attention because they can form an effective cathode–electrolyte interphase (CEI) layer, which delays the electrolyte decomposition.^{20–22} Although individual modifications can be used for improving the cycling behavior of each electrode, they increase the cost of LMBs, which can be a critical challenge for the overall future market of LMBs.

Recently, it has been established theoretically that it is possible to simultaneously enhance the overall performance of LMBs by employing dual effect additives for both Li metal and NCM811. Therefore, the application of various electrolyte additives has been proposed to restrain undesired surface

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Poly(Imide) Separator Functionalized by Melamine Phosphonic Acid for Regulating Structural and Thermal Stabilities of Lithium-ion Batteries

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ABSTRACT

As the energy density of lithium-ion batteries (LIBs) continues to increase, various separators are being developed to with the aim of improving the safety performance. Although poly(imide) (PI)-based separators are widely used, it is difficult to control their pore size and distribution, and this may further increase the risk associated. Herein, a melamine phosphonic acid (MP)-coated PI separator that can effectively control the pore structure of the substrate is suggested as a remedy. After the MP material is embedded into the PI separator with a simple one-step casting process, it effectively clogs the large pores of the PI separator, preventing the occurrence of internal short circuits during charging. It is anticipated that the MP material can also suppress rapid thermal runaway upon cycling due to its ability to reduce the internal temperature of the LIB cell caused by the desirable endothermic behavior around 300°C. According to experiments, the MP-coated PI separator not only decreases the thermal shrinkage rate better than commercial poly(ethylene) (PE) separators but also exhibits a desirable Gurley number (109.6 s/100 cc) and electrolyte uptake rate (240%), which is unique. The proposed separator is electrochemically stable in the range 0.0–5.0 V (vs. Li/Li⁺), which is the typical working potential of conventional electrode materials. In practice, the MP-coated PI separator exhibits stable cycling performance in a graphite–LiNi_{0.83}Co_{0.10}Mn_{0.07}O₂ full cell without an internal short circuit (retention: 90.3%).

Keywords : Lithium-ion batteries, Separator, Poly(imide), Melamine phosphonic acid, Electrochemical performance

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1. Introduction

The use of thermally stable separators in lithium-ion batteries (LIBs) has increased in recent years, given the continuing attempts to increase their energy density [1–4]. Many advanced electrode materials must offer a high specific capacity and working potential [5–10]; this further increases the need to ensure the safety of high-density LIBs as they may cause severe accidents with abnormal use [11–15]. Therefore, to improve the safety of LIBs, the use of separators has garnered significant attention over the

past few years.

The separator is located between the electrodes to prevent electron migration, and several insulating materials have been developed [16–18]. The most frequent types are made from poly(olefin)-based poly(ethylene) (PE) and poly(propylene) (PP), as they provide effective insulation ($\sim 10^{14}$ Ω cm) and can be mass-produced [19–24]. Moreover, as these polymeric materials are cost-effective compared to other insulating materials, poly(olefin)-based materials have served as a core separator since the first commercialization of LIBs [25–27].

Although poly(olefin)-based materials, especially in PE, have been used in LIBs, their relatively unstable thermal behaviors still remain a limitation. Note that the cells could be ignited by abnormal use, then the temperature of the cells is rapidly increased by

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Fluoride scavengeable Sb_2O_3 -functionalized poly(imide) separators for prolonged cycling of lithium-ion batteries†

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Nanosize-controlled antimony oxides (Sb_2O_3) that can effectively scavenge fluoride species in a cell are incorporated into a PI separator to regulate its porous structure. The incorporation of the Sb_2O_3 layer onto the PI separator surface prevents the internal short circuit and efficiently removes fluoride species via chemical scavenging reactions, thereby resulting in stable cycling behaviors upon cycling.

Owing to the increasing demand for large-scale energy power sources, many advanced electrode materials with high energy densities have been developed for lithium-ion batteries (LIBs).^{1,2} Although advanced electrode materials have been developed over the past few decades as the energy density of LIBs has dramatically increased, ascertaining the safety of LIBs has become another critical issue in the energy society.^{3,4} Among the components of LIBs, the separator placed between the cathode and anode plays a significant role in physically separating the cathode and anode, ensuring the safety of LIBs.⁵ The separator should be an ionic conductor and an electronic insulator that only allows Li^+ migration and does not permit electron transport between the electrodes. In this regard, electronic insulating materials such as poly(ethylene) (PE)⁶ and poly(propylene) (PP)⁷ have been intensively developed for LIBs. The most frequently used separators are PE-based polyolefin-type polymers: although the intrinsic properties of PE separators depend on the production method, such as

whether it is dry or wet, their desirable electrochemical performance at low cost permits them to be primary separators in LIBs.⁸ Nevertheless, the relatively low thermal stability of conventional PE separators is a critical limitation for employing LIBs with high energy densities: if the cells are used abnormally, thermal runaway (which indicates a drastic increase in the cell temperature) can occur only with trivial triggers.⁹ When the cell temperature rapidly rises and reaches 120 °C, the PE separator begins to shrink owing to the phase transition.¹⁰ Note that the role of PE is to separate the electrodes; however, avoiding short circuits between the cathode and anode above 120 °C is not controllable owing to drastic shrinkage behaviors, which accelerates an exponential temperature increase of the cell under a thermal runaway situation.¹¹ Therefore, the need to develop functional materials that do not deform when used as separators, even at high temperatures, has constantly increased.

In this study, a functionalized poly(imide) (PI)-based thermally stable separator was developed for LIBs (Fig. 1a). Note that PI-based materials are thermally stable over 300 °C,¹² making them robust against phase melting and thermal decomposition up to 300 °C. However, because state-of-the-art PI-based separators are composed of abundant porous structures, they cannot entirely prevent internal short circuits even at the initial cycle. To overcome these limitations, we incorporated antimony oxide (Sb_2O_3) to regulate the internal short circuit that arises from the abundant pores in the PI separators, which ensures stable cycling performance of the cell. Moreover, Sb_2O_3 can bind well to the halogen species that remains in the cell via chemical reaction¹³ (which can prevent the dissolution of metal components that exist in typical cathode materials),¹⁴ thus, employing the Sb_2O_3 is expected to improve cycling retention. Moreover, it has been reported that such a scavenging reaction is typically considered an endothermic reaction,¹⁵ implying that incorporating the Sb_2O_3 may reduce the drastic increase in the cell temperature.

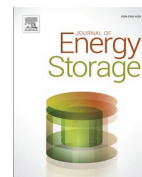
After coating Sb_2O_3 on the PI separator, its surface morphology was analyzed by scanning electron microscopy (SEM), as

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Research Papers

Sustainable cathode-electrolyte interphases of nickel-rich layered oxides in-situ functionalized by lithium fluoride abundant layers for lithium-ion batteries

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ABSTRACT

Although Nickel-rich cathodes have been noted as key materials for electric vehicles, their reliable high-temperature performance is considered the primary bottleneck for future applications. Herein, we propose 1H, 1H, 5H-octafluoropentyl 1,1,2,2-tetrafluoroethyl ether as an interface stabilizing additive for Ni-rich cathode, which can offer cathode-electrolyte interphase layers. It is anticipated that fluorine-abundant proposed additives can form lithium fluoride-based cathode-electrolyte interphase layers through electrochemical oxidation, making the cathode-electrolyte interphase layers robust and durable due to their improved mechanical properties. In the analyses, it is observed that the suggested additive provides 5.5 nm-based cathode-electrolyte interphase layers at the cathode interface by electrochemical oxidation. In terms of cycling performance, the additive-contained electrolytes reveal prolonged cycling retention (62.2%), whereas the standard electrolyte displays only 40.7% retention because electrolyte decomposition is not managed in the absence of the additive. Further, post-analyses indicate that durable lithium fluoride-based cathode-electrolyte interphase layers remain on the recovered cathode, whereas most of the surface is covered by decomposed adducts in the cycled cathode in the presence of the standard electrolyte.

1. Introduction

Since their commercialization as power supply sources in 1991, lithium-ion batteries (LIBs) have demonstrated an effective widening of their application range to medium and large devices. The development of new applications requires higher energy densities than those of conventional LIBs; thus, enhancing the energy density of LIBs has emerged as an enormous challenge [1,2]. The relationship between the energy density and electrode materials in LIBs has been well established; consequently, significant research has been undertaken over the last few decades to develop advanced electrode materials [3–6].

Currently, significant research and development are being conducted on cathode materials based on Ni-rich layered materials ($\text{LiNi}_x\text{Co}_y\text{Mn}_{1-x-y}\text{O}_2$, $x \geq 0.6$, Ni-rich NCM). It is widely recognized that they enable an increase in the specific capacity when the Ni composition is over 60% [7–9]. Ni-rich NCM materials are derived from conventional lithium cobalt oxide (LiCoO_2) by partial replacement of the Co with Ni [10,11].

The working potential of $\text{Ni}^{2+}/\text{Ni}^{3+} \leftrightarrow \text{Ni}^{4+}$ is low compared to that of Co species [12]; therefore, specific capacity could be designated by controlling the Ni composition. Therefore, NCM has attracted significant attention because it can offer an increased specific capacity by increasing the amount of Ni instead of Co. Nevertheless, NCM materials face challenges in overcoming critical bottlenecks, particularly interfacial instability. NCM materials are composed of abundant Ni species, and even if abundant Ni species contribute to increasing the specific capacity, thermodynamically unstable Ni^{4+} is always retraced to stable Ni^{2+} species, and this intrinsic nature severely triggers electrolyte decomposition at the interface by taking electrons from the electrolyte [13]. Such undesired reactions are considerably accelerated at high temperatures; therefore, NCM cathode materials experience poor cycling retention, especially at high temperatures. Furthermore, NCM cathode materials suffer from severe microcracking due to low particle strength during charging and discharging; once a microcrack is formed at the boundaries of secondary particles, additional electrolyte

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RESEARCH ARTICLE

Stabilizing Interfacial Behaviors of Ni-rich Cathodes by Upcycling of Oyster Shells Waste

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Layered lithium oxides (LNCM), with over 60% Ni, are promising cathode materials for electric vehicles due to their high capacity. However, reliable cycling retention cannot easily be achieved owing to the instability of the interface. Here, recycled oyster shell (OS) is employed as an efficient precursor for the surface modification of LNCM cathodes; subsequently, Ca-based artificial cathode–electrolyte interphases (CEI) are obtained at the LNCM interfaces through a simple calcination process. When the pulverized OS is calcined with LNCM cathode materials at elevated temperatures, CaCO_3 is converted to CaO via thermal decomposition at the LNCM interface, which effectively protects the unstable interface. Microscopic analyses indicate the nanodomain island-type CEI layers embedded after OS calcination. The OS-coated LNCM cathode materials increase the cycling retention after 100 cycles (93.8% vs. 62.7%) and decrease the internal cell pressure by preventing electrolyte decomposition. Incorporating Ca into the LNCM cathode materials also inhibits microcrack formation because of the improvement in particle hardness and the remarkable decrease in the irreversible dissolution of transition metals from LNCM cathodes, considering that Ca plays a crucial role in scavenging fluoride species. Consequently, recycling OS as a functional LNCM precursor is an innovative approach for enhanced cycling retention.

density than that provided by conventional LIBs for small-scale devices; therefore, considerable research has been conducted to develop advanced electrode materials with high voltage and specific capacity.^[4–6] Notable electrode materials that offer high energy density can be divided into two categories: 1) high-working voltage and 2) high-capacity materials, because the energy density is proportional to the voltage and specific capacity of the cell. In this regard, 5 V-class cathode materials, such as lithium-rich layered oxide,^[7,8] and lithium nickel manganese oxide,^[9,10] have attracted considerable interest, considering that their average voltages are higher than that of conventional cathode, lithium cobalt oxide (LCO). Cathode materials with larger specific capacities than that of conventional LCO have also been widely investigated, particularly, layered oxides with high Ni contents (Ni-rich LNCM, $\text{LiNi}_x\text{Co}_y\text{Mn}_z\text{O}_2$).^[11,12] Note that the specific capacity can be increased when additional Ni replaces Co at the layered

1. Introduction

The burgeoning supply of electric vehicles worldwide has rapidly increased the demand for lithium-ion batteries (LIBs), a major power source for electric vehicles. LIBs can convert and store electrical energy as chemical energy, and their market has expanded from small- to large-scale devices in the past several years.^[1–3] New applications of LIBs require a substantially higher energy

sites because Ni exhibits lower working potential than Co if the cell is de-lithiated at identical potentials (≈ 4.3 V, vs. Li/Li^+).^[13] In this context, replacing Co with a large amount of Ni is apparently beneficial in terms of energy density. Accordingly, LNCM cathode materials containing more than 60% Ni have gained outstanding attention as advanced cathode materials over the past several decades.^[14]

Nevertheless, high-Ni LNCM cathode materials present several critical problems owing to their unstable cycling behavior. Generally, LNCM cathode materials are prepared via LNCM–OH calcination with Li sources (LiOH or Li_2CO_3) in slight excess. Although excess Li is necessary to promote the complete dehydration of LNCM–OH, unreacted excess Li remains on the LNCM surface.^[15] This residual Li decomposes into gaseous components via an electrochemical reaction at ≈ 4.0 V (vs. Li/Li^+).^[16] thereby decreasing the cell safety via sudden swelling of the cell. In addition, when Co is replaced with a large amount of Ni in LNCM cathode materials, the amount of unstable Ni^{4+} increases during electrochemical charging.^[17] Particularly, Ni^{4+} is highly unstable because it tends to be easily reduced; therefore, the electrolyte easily decomposes at the LNCM interface because of prompting electron transfer reactions between Ni^{4+} and the electrolyte, thus forming unstable cationic intermediates from the decomposition of the electrolyte.^[18] When these parasitic

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Design Principles for Moisture-Tolerant Sulfide-Based Solid Electrolytes and Associated Effect on the Electrochemical Performance of All-Solid-State Battery

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ABSTRACT

The grave concern on the safety of Li-ion batteries adopted in commercial electrical vehicles pushes an urgent demand for developing safer all-solid-state batteries (ASSBs), where ion-conducting solid electrolytes play pivotal roles. Much higher conductivity and more ductile nature of sulfide-based electrolytes offers great advantages over conventional oxide materials in terms of manufacturing process difficulty and the battery performance. However, instability of sulfide materials towards atmospheric moisture results in the substantial degradation in the ionic conductivity and the release of hazardous gas. After over a decade of intensive research, various customized strategies based on the specific design rules were developed for each electrolyte to tackle this crucial issue. However, in most cases a moisture tolerance was endowed only after compromising its vital ionic conductivity to some extent. Nevertheless, the actual applications of sulfide electrolytes to ASSBs often lead to improved battery performance by virtue of the interfacial stabilization between oxide-based cathode materials and sulfide-based solid electrolytes. Therefore, it is essential to fully comprehend the critical factors of each atmospheric stabilization technology that potentially affects the eventual battery performance. Herein, we go over the current status of state-of-the-art moisture-stabilizing technologies for each sulfide-based solid electrolyte, summarizing the major effect of each technology on the various aspect of the electrochemical performance upon application. We believe that this review will contribute to achieving effective moisture-stabilization of sulfide-based solid electrolytes, catalyzing the successful commercialization of sulfide-based ASSBs.

Keywords : Sulfide-based solid electrolytes, All-solid-state batteries, Moisture-tolerance, Ionic conductivity, Design principles

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1. Introduction

Despite a good deal of acclaims for their successful application to commercial electric vehicles (EVs) and

energy storage systems (ESSs), lithium-ion batteries (LIBs) have still suffered from apparent limitations related to safety and practical energy density achievable, which have propelled more than a decade of intensive research on the next-generation batteries [1–4]. Among many advanced LIBs developed so far, all-solid-state batteries (ASSBs) are considered as one of the most viable candidates particularly owing to their high safety features [4–6]. To date, two types of solid electrolytes have been primarily employed in ASSBs, i.e., oxide- and sulfide-based materials. Owing to their mechanically robust particle strength,

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A Dendrite-Free Lithium Metal Battery by Incorporating Poly(dimethylsiloxane) Layer onto the Porous Three-Dimensional Copper Current Collector

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Abstract

As an anode, lithium metal electrode is one of the most promising candidates for lithium batteries because of their theoretically high specific capacity and low redox potential. However, lithium dendrites are formed during the cycle of lithium deposition and dissolution on the copper current collector during charging and discharging, threatening the stability and durability of lithium metal batteries (LMBs). In this study, a three-dimensional (3D) porous copper coated with a polymer layer (polydimethylsiloxane, PDMS) is used as a current collector to ensure high stability. A PDMS-coated 3D porous current collector is prepared by Cu electrodeposition using hydrogen bubbles as a template followed by a PDMS coating. PDMS coating is performed by spin coating of PDMS on a slide glass and transferring it to the current collector. The 3D current collector functionalized by PDMS offers prolonged lifespans in both Li deposition/dissolution and intercalation of the lithium iron phosphate cathode, as the functionalized PDMS-coated 3D porous current collector effectively prevents the growth of Li dendrites at the interface.

Keywords Lithium metal anode · 3D current collector · Cu foam · Polydimethylsiloxane · Dendrite free · Lithium metal battery

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Introduction

Interest in renewable and sustainable energy storage and conversion technologies has increased as a consequence of global warming. Secondary batteries, which can be charged and discharged semi-permanently using lithium or zinc redox processes, are an efficient energy storage technology [1–3]. This battery technology is receiving significant attention due to its high power density and low storage cost, which resolve the huge energy production fluctuations of new and renewable energy technologies. However, except for mobile devices and automobiles, battery energy density does not meet the needs of large-scale industries. In this regard, the lithium metal anode has received interest as a next-generation battery technology because it has a high theoretical capacity of 3860 mA h g^{-1} and the lowest redox potential of -3.04 V vs. a standard hydrogen electrode [1–7]. However, the formation of lithium dendrites while repeating the deposition–dissolution process diminishes the long-term performance of the battery and increases the risk of explosion in lithium metal batteries (LMBs) [1–8].



Multiplexed detection of single-point mutations in unprocessed human serum with SERS-integrated PCR

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ABSTRACT

Early detection and monitoring of tumor mutations is an important area of research in diagnostics. Polymerase chain reaction (PCR) is considered the gold standard for monitoring tumor mutations. Recently, integration of PCR with SERS has led to enhanced sensitivity and multiplexity of methods for monitoring pathogens and mutations. However, limitations on increasing the number of targets that can be detected using a single set of reaction still exist. The number of mutations that can be analyzed in a single reaction is influenced by the interaction among different fluorophores. For simultaneous detection of more than three mutations with SERS, complicated extraction, and subsequent statistical analysis is required. Here, we present a SERS Hairpin-blocked Integrated PCR (SHIP) system, which integrates PCR with SERS using hairpin-shaped blocking DNA to prevent false positive enhancement of SERS intensity. SHIP enables simultaneously detection of up to four different KRAS single-point mutations in unprocessed serum. The SHIP system holds a great potential for multiplexed detection of mutations and pathogens for diagnostic purposes.

1. Introduction

Detection of mutations in a multiplexed and accurate manner is of great importance for early diagnosis and monitoring of cancer. Some precancerous mutations are related to aging and remain benign whereas others may be the first step in the development of cancer [1]. Different tumors exhibit a high diversity of mutated genes, which are directly associated with the cancer type [2]. Detection of various mutations in a single test can facilitate an early diagnosis of the disease and improve control the disease progression better, leading to the designing of more effective and personalized treatments [3,4].

For personalized medication, it is essential to identify the different mutations present in a tumor. The presence of specific gene mutations determines the characteristics of the tumor, such as its aggressiveness and potential to disseminate in the body. Determining the type of mutation present in the tumor is of great importance for a better prognosis and for controlling the progress of the disease. Detection of certain mutations that are considered potential druggable targets is essential for the development of more precise treatments [5].

KRAS, which encodes a small G-protein in the EFGR pathway, is one of the most extensively studied proto-oncogenes [6,7]. KRAS mutations

are present in 25% of human cancers [8], with a particularly high incidence in colon and pancreas cancer [9,10]. The most frequent KRAS mutations are G12D, G12V, G12S, and G13D, the presence of which can provide information about the origin and progression of the tumor [11]. Simultaneous monitoring of these four mutations in a single test could be very useful.

Over the past several decades, the accuracy of molecular diagnostic methods has improved considerably owing to the use of well-known techniques, such as immunoassay [12] and polymerase chain reaction (PCR) [13]. The integration of these techniques with advanced techniques from other fields enables the development of molecular diagnostic systems with potential clinical applications [14,15].

PCR was originally designed to amplify DNA fragments [16]. Incorporation of new technologies into the basic PCR method has led to the evolution of more precise methods, such as qPCR or digital PCR [17]. In qPCR, the real-time detection of nucleic acids is achieved by associating a fluorophore with a probe specific to the target sequence [18]. However, the light sources used for detecting the fluorophores pose a limitation for multiplexed detection because only one fluorophore associated with each light channel can be detected simultaneously.

To overcome the limitation of multiplexed detection, PCR has been

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Integration of wide-field imaging system with droplet microfluidics for monitoring living bacteria

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ABSTRACT

Droplet microfluidics has been widely used to analyze chemicals and biological reactions at the single-molecule level. Microscopic systems are commonly used for imaging and analyzing droplets using high-magnification objective lenses. However, these systems are expensive, complex, and large, limiting the high-throughput characterization of droplets in reactions targeting disease-related biomarkers, including nucleic acids, proteins, and pathogens. Another concern is the time gap between droplet analysis within a microfluidic channel and imaging chamber, which can cause discrepancies in reaction time between droplets. To address this issue, we introduce the droplet analysis system for vast imaging and statistical tool (DropVIST)—a wide-field imaging system integrated with droplet microfluidics for rapid and accurate droplet analysis. DropVIST can detect eight differently colored droplets simultaneously using a wide-field imaging system and dFinder software, which can quantify the reacted droplets. The smallest droplet diameter for detection was 30 μm , and the maximum detection area was 201.84 cm^2 , suggesting that the system can theoretically analyze 3103,377 droplets in real-time. We validated the monitoring performance of DropVIST using clinical isolates of five types of living pathogenic bacteria and compared this with conventional approaches, including the microbroth dilution method and colony-forming unit assay. This platform provides a rapid, simple, and accurate tool for monitoring living bacteria at the single-cell level.

1. Introduction

Droplet-based digital microfluidics involves the manipulation and characterization of individual droplets [ranging from nanoliters (10^{-9} L) to picoliters (10^{-12} L)] generated within microchannels [1–4]. Biological samples can be partitioned into individual droplets containing a single molecule of interest, such as nucleic acids, proteins, or pathogens, and then incubated for signal amplification within a picoliter-sized micro-reactor [5–7]. A positive result (1) indicates the presence of the target molecule, whereas a negative result (0) indicates the absence of the target molecule in the droplet. By categorizing the results as positive or negative, absolute quantitative data can be obtained without a standard curve or controls [8–10]. This digital analysis offers several advantages, including lower false negatives/positives associated with the increased signal-to-noise ratios at the single-molecule level in conventional approaches that use samples of milliliter (10^{-3}) to microliter (10^{-6}) volumes [11–13]. Therefore, digital analysis has been incorporated with

various next-generation sequencing technologies, such as droplet digital polymerase chain reaction (PCR) and digital enzyme-linked immunosorbent assay (ELISA) [14–17].

Current digital approaches use commercial microscope imaging systems, such as optical, fluorescence, and confocal microscopes, to obtain high-resolution images of droplets and software to extract quantitative data [18–21]. Although microscopic imaging systems provide high-resolution images, they are costly, only allow endpoint analysis, and have a narrow field of view when using high-magnification objective lenses, which limits the analysis of wide-field images or large quantities of samples, leading to longer experimental times and an increased demand for human resources. Image stitching resolves the limited field of view issue by combining multiple images, which improves sample processing speed [22,23]. However, the technique has several limitations. It is difficult to accurately identify matching points for stitching when droplets move and requires auto-focusing processes to analyze each image for stitching, which is time-consuming and

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Review

Droplet microfluidics for single-molecule and single-cell analysis in cancer research, diagnosis and therapy



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ABSTRACT

Genetic and functional heterogeneity of tumor cells represents major obstacles to cancer research, detection and effective treatment. In order to develop new therapeutic approaches in an era of personalized medicine, it is important to understand the functional characteristics of DNA, RNA, and proteins at the single-molecule level in individual cancer cells. Droplet microfluidics has emerged as a new tool that offers advantages in analyzing single molecules and single cells for high-throughput analysis with exceptional sensitivity. In this review, we highlight some recent reports that employed droplet microfluidics for cancer research, diagnostics, and therapeutics, and offer a view on future applications.

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1. Introduction

Molecular and cellular heterogeneity in malignant tumors represents major hurdles to developing effective cancer therapy [1], so accurate analysis of individual biomolecules, such as DNA, RNA, and proteins, in cancer can help to improve understanding of the development, the progression and the classification of tumor types at the cellular and genetic levels, and that may further aid the

development of new diagnostic and therapeutic approaches [2]. Currently, much of our knowledge of cancer biology is based on information obtained from bulk experiments using traditional population-averaged approaches, such as Western blotting, proliferation assays, DNA sequencing or cytotoxicity assays [3]. While these approaches have been useful for understanding features of cancer, key information, such as molecular distributions, functional variations, and drug-target interactions, may be hidden at the levels of the single molecule and the single cell. Also, isolation and analysis of single molecules and single cells in a high-throughput manner with large sample volumes are essentially impossible to implement using the traditional methods. There is also a pressing need for new diagnostics that can detect rare cancer

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Tailored synthesis of pH-responsive biodegradable microcapsules incorporating gelatin, alginate, and hyaluronic acid for effective-controlled release

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ABSTRACT

In response to escalating environmental concerns and the urgent need for sustainable drug delivery systems, this study introduces biodegradable pH-responsive microcapsules synthesized from a blend of gelatin, alginate, and hyaluronic acid. Employing the coacervation process, capsules were created with a spherical shape, multicore structure, and small sizes ranging from 10 to 20 μm , which exhibit outstanding vitamin E encapsulation efficiency. With substantial incorporation of hyaluronic acid, a pH-responsive component, the resulting microcapsules displayed noteworthy swelling behavior, facilitating proficient core ingredient release at pH 5.5 and 7.4. Notably, these capsules can effectively deliver active substances to the dermal layer under specific skin conditions, revealing promising applications in topical medications and cosmetics. Furthermore, the readily biodegradable nature of the designed capsules was demonstrated through Biochemical Oxygen Demand (BOD) testing, with over 80 % of microcapsules being degraded by microorganisms after one week of incubation. This research contributes to the development of responsive microcapsules and aligns with broader environmental initiatives, offering a promising pathway to mitigate the impact of microplastics while advancing various applications.

1. Introduction

Microencapsulation is a versatile technique that encapsulates active materials to protect and deliver them to target sites, enabling optimal efficacy of the core ingredients while facilitating controlled release [1–5]. In recent years, the development and optimization of responsive delivery microcapsules have gained significant prominence owing to their vast potential applications in various fields, such as targeted drug delivery, agriculture, cosmetics, and environmental protection [6–9]. Within the realm of stimuli-responsive microcapsules, pH-responsive microcapsules have emerged as noteworthy candidates because of their ability to release their encapsulated contents in response to environmental pH changes [10]. This trait is particularly valuable in

applications where localized pH alterations, such as those in the gastrointestinal tract or certain skin conditions, can trigger the release of a designated agent [11–14]. Consequently, it is paramount to develop new materials that are biocompatible and demonstrate heightened responsiveness, especially with capabilities that activate in targeted scenarios. This advancement is vital for enhancing targeted delivery, reducing systemic side effects, and improving the overall efficacy and safety of microencapsulation technologies. In fact, the functionality and physicochemical attributes of these microcapsules are intrinsically influenced by the selection and combination of the wall materials.

In this context, the distinct and synergistic advantages of gelatin, alginate, and hyaluronic acid are capitalized to synthesize a superior microcapsule system. These natural polymers provide the material with

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Review

생분해성 플라스틱 분해 조절을 위한 미생물 및 효소 기술

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인천대학교 바이오 플라스틱 센터, *인천대학교 기초과학연구소
(2024년 5월 24일 접수, 2024년 6월 2일 수정, 2024년 6월 7일 채택)

Microbial and Enzymes That Can Regulate Plastic Degradation

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초록: 생분해성 플라스틱은 기후변화 대응의 일환 그리고 탄소중립 실현을 위한 기존 난분해성 플라스틱 대체제로 개발되어 기술적, 산업적 측면에서 빠르게 성장하고 있다. 그러나, 산업적 퇴비화 조건과 달리 일반적인 환경은 생분해성 플라스틱이 충분한 분해속도를 가지기에 열악한 조건으로 기존 생분해성 플라스틱의 분해 속도 조절에 대한 다양한 요구가 이루어지고 있는 실정이다. 따라서, 최근 생분해성 플라스틱의 호기 및 혐기 조건에서의 분해 가속화를 위한 다양한 연구가 활발히 이루어지고 있으며, 특히 생분해 미생물 및 효소 발굴과 이의 개량에 대한 연구가 활발히 이루어지고 있다. 본 논문에서는 최근 이루어지고 있는 생분해성 플라스틱 분해 미생물 및 효소의 개량에 대한 연구에 대한 정리를 통해, 생분해성 플라스틱 분해조절을 위한 정보를 제공하고자 한다.

Abstract: Biodegradable plastics have been developed as a replacement for non-degradable plastics to respond to climate change and achieve carbon neutrality and are growing in technological and industrial terms. However, the biodegradability of biodegradable plastics does not meet what is required from user aspects, and various demands are being made to control the decomposition speed and initiating time points. Therefore, multiple studies have recently been actively conducted to accelerate the decomposition of biodegradable plastics under aerobic and anaerobic conditions, particularly research on the discovery and improvement of biodegradable microorganisms and enzymes. In this paper, we aim to provide information for controlling the decomposition of biodegradable plastics by summarizing recent research on microorganisms and the enzymes.

Keywords: biodegradable polymer, biodegradable plastic, microbial, enzyme, plastic digestion.

서 론

1907년에 최초로 합성된 플라스틱 고분자는 베이클라이트(Bakelite)로 세계 2차 대전 이후 군 외부에서 플라스틱이 널리 사용되면서 플라스틱 생산의 급속한 성장을 이루었다.^{1,2} 플라스틱은 인공적으로 합성된 유기 폴리머로 정의되며, 주로 석유에서 유래한 비생분해성 물질로 낮은 열 및 전기전도

도, 높은 내구성, 우수한 기계적 성질과 합리적인 가격을 제공한다.³ 석유화학 플라스틱은 1930~1940년부터 상업적으로 이용되기 시작해 1950년대부터 대량 생산에 이르러, 지난 50년간 20배 증가하였다.⁴ 2019년 연간 플라스틱 생산량은 3억 5900만 톤으로 전세계 누적 플라스틱 생산량은 2050년 말까지 260억 톤에 이를 것으로 예상된다.⁵ 석유계 플라스틱의 뛰어난 기계적 물성, 가공성, 경량성은 소비재 제품의 성능을 단기간에 향상시켜주며 우리에게 사회적, 경제적 이익을 안겨주었다.⁶ 현재 석유계 플라스틱은 패키징, 전자, 의료, 미용 등 다양한 산업에서 경량 및 유연성을 조절할 수 있는 범용성을 가진, 필수불가결한 소재이다.⁶

하지만, 혁명적이고 편리한 소재였던 플라스틱은 높은 안

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Advancing organic electronics: Achieving reliable synthesis of conjugated polymers with various carrier polarities using a continuous flow reactor

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ABSTRACT

Donor-acceptor (D-A) conjugated polymers often exhibit excellent performance in electronic and photonic applications, however, their synthesis remains a hurdle to achieving their wide-spread commercialization. While scalable and reproducible flow syntheses have emerged as promising candidates to address this issue, they have only been applied to a narrow scope of polymer types. In this study, we explore the synthesis of six different D-A conjugated polymers in a continuous flow reactor to determine how reaction and polymer parameters influence the degree of polymerization (DP) and quality of the resulting polymers. We found that we could successfully synthesize *p*-type, *n*-type, and ambipolar D-A conjugated polymers with a single set of reaction parameters. Only the reaction time required optimization to achieve high-DPs. Importantly, we found that we could achieve comparable DPs to batch reactions, but with higher yields and much shorter reaction times. High-quality polymers were also obtained, which was confirmed with optical and electrochemical properties measurements and by their performance in organic field-effect transistors. We expect this work to further establish flow synthesis as an ideal method to make D-A conjugated polymers, which may help facilitate their commercialization in high-performing organic devices.

1. Introduction

The donor-acceptor (D-A) strategy is a popular design approach for conjugated polymers, which involves the incorporation of alternating electron-rich donor and electron-poor acceptor units into the polymer backbone [1–6]. The choice of donor and acceptor units can be tailored to tune the energy bandgap of the polymer, which can affect its electronic and optical properties [1–6]. D-A conjugated polymers typically outperform other types of conjugated polymers used in organic devices, such as organic photovoltaics, organic light-emitting diodes, and organic field-effect transistors [7–9]. Therefore, research in this field has been focused on developing new D-A conjugated polymers with optimized donor and acceptor units, as well as backbone structures, to further enhance their performance in organic devices [1–9].

One of the biggest limitations of D-A conjugated polymers is the reproducibility of their syntheses. As the molecular weight is a key factor that influences material properties and the performance of polymers in organic devices [10], it is important to synthesize conjugated polymers

with controlled and reproducible molecular weights. The most commonly used synthetic method for D-A conjugated polymers is the Stille polycondensation, which is typically carried out in batch reactors. However, this approach leads to significant batch-to-batch variation in molecular weight and molecular weight dispersity (*Đ*). Therefore, the development of new synthetic methods to address this reproducibility issue represents an important step towards commercializing these materials and advancing their use in high-performing organic electronic and photonic devices.

Flow chemistry is known to provide high reproducibility and selectivity for reactions, and has been applied to the synthesis of several D-A conjugated polymers (Fig. 1) [11–17]. In 2012, Seyler et al. reported the flow synthesis of a thieno[3,4-*b*]thiophene- and benzodithiophene- based copolymer (PTB) [12]. In subsequent studies, the potential for large-scale (>100 g/day) conjugated polymer synthesis was demonstrated, and the quality of polymers was also demonstrated by applying them to organic electronic and photonic devices [13]. Since then, the focus of the field has largely focused on expanding the scope of high-performance D-

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Depolymerization of Polymethacrylates with Ball-Mill Grinding

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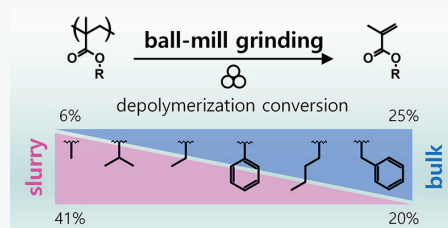
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Supporting Information

ABSTRACT: In this report, we demonstrate that polymethacrylates can undergo appreciable depolymerization, with mild conditions and without reactive end groups, using ball-mill grinding (BMG). For instance, the important commercial polymer, poly(methyl methacrylate) (PMMA) underwent up to 41% depolymerization to monomer within 8 min of grinding. We explored the factors that influence the depolymerization conversion and found temperature (heating to only 43 °C), addition of a small amount of liquid (μL of *tert*-butyl alcohol), and ball number/size to be the most important parameters. We also applied this method to other polymethacrylates and found the pendant substituent to influence the extent of depolymerization.

Addition of a liquid was found to reverse the effects of the substituent that were observed with bulk grinding. For instance, among the polymethacrylates studied, PMMA had the lowest depolymerization conversion in bulk and one of the highest depolymerization conversions in a slurry. Notably, the molecular weight of the residual polymer is remarkably low, ca. 1 kDa, demonstrating the excellent degradation performance of BMG. Overall, this work provides important mechanistic insight into depolymerization reactions under ball-milling conditions and represents an important step in expanding BMG as a tool for the chemical recycling of polymethacrylates.



INTRODUCTION

Chemical recycling to monomer is an important form of recycling that can contribute to a cyclic polymer economy.^{1–4} While these techniques are not energetically accessible for many poly(alkenes), poly(methyl methacrylate) (PMMA) is a promising candidate.¹ Pyrolysis typically requires temperatures in excess of 300 °C and produces monomer (methyl methacrylate, MMA) in addition to other biproducts.^{5,6} In recent years, considerable effort has been devoted to developing milder chemical recycling methods for PMMA and other polymethacrylates, such as reverse controlled polymerization.⁷

In the reverse controlled polymerization of polymethacrylates, reactive chain ends are cleaved, generating macroradicals that can depolymerize. The reactive chain ends are most commonly halogen chain ends from atom transfer radical polymerization (ATRP)^{6,8–12} or iodine transfer polymerization (ITP),¹³ and thiocarbonylthio chain ends from reversible addition–fragmentation chain-transfer (RAFT) polymerization.^{14–19} Depolymerization conversions up to 86% and 92% have been reported for PMMA and poly(*n*-butyl methacrylate) (PnBMA), respectively.¹⁴ However, these methods typically require high temperatures, dilute conditions, use of catalysts, specific end-groups, and/or long reaction times (Figure 1A). Conversion of the chain ends to other reactive groups has facilitated high-yielding depolymerizations (>90% depolymerization of PMMA) in bulk,^{20,21} although the need for high temperatures (>150 °C) and specific end-groups remain limitations.

Ball-mill grinding (BMG) is a popular technique used for polymer synthesis,^{22–29} polymer degradation,^{30–37} and other mechanochemical transformations.^{38–43} Early studies pertaining to the BMG of PMMA were interested in the macroradicals that were generated upon chain scission.^{44–46} Chain scission necessitates a reduction in the molecular weight, which will continue to decrease until reaching a limiting value (often referred to as the limiting molecular weight, M_{lim}). The M_{lim} for PMMA is dependent on the grinding conditions, but has been reported to be as low as 5 or 6 kDa.^{45,47,48} Thus, ball-milling of PMMA typically yields moderate to low molecular weight PMMA without depolymerization (Figure 1B). The use of mechanochemical techniques for chemical recycling to monomer is a developing area that may provide mild methods for depolymerizing polymethacrylates.⁴⁹ We recently reported the use of BMG to degrade poly(α -methylstyrene) (PMS).⁵⁰ Up to 64% depolymerization of PMS was observed after only 8 min of grinding, with negligible bulk heating. We also subjected PMMA to our optimized conditions, but obtained only 4% depolymerization after 8 min of grinding (Figure 1B). The significant reduction in the extent of depolymerization was

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Progress toward sustainable polymer technologies with ball-mill grinding

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ABSTRACT

The ball-mill grinding (BMG) of polymers has a long history, starting with Staudinger showing in the 1930s that polystyrene undergoes chain scission upon ball milling. However, BMG has significantly expanded from being used solely for polymer degradation to a synthetic tool for a range of applications only in the last decade. Now, BMG has emerged as a promising mechanochemistry technique for several critically important polymer technologies, such as recycling and upcycling, and often provides novel or enhanced mechanochemical reactivity. As a solid-state technique in which solvents are often minimized or eliminated, BMG provides a greener and more sustainable route to various applications. Also, in contrast to many other mechanochemistry techniques that are commonly employed with polymers, BMG has the potential to be scaled to industrially relevant levels. In our review, we provide an extended and deep overview of the phenomena that occur when polymers are subjected to BMG and show how these phenomena can be exploited for various applications. We treat particularly technologies that, especially in the context of our current plastic pollution crisis, are relevant to trending topics in the field of polymer science, such as polymer degradation, chemical recycling, recycling, and upcycling. Other important topics covered in this review include the mechanical activation of responsive polymers, by the use of mechanophores or by exploiting the reactivity of the reactive intermediates generated during chain scission, and polymer-assisted grinding, where polymers serve as additives or reagents to aid in mechanochemical syntheses or other processes.

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Abbreviations: AA, acrylic acid; ABS, acrylonitrile butadiene styrene; Ad, adamantane; AIE, aggregation-induced emission; BAPy, 1-(bromoacetyl)pyrene; BMG, ball-mill grinding; BPA, bisphenol A; BHT, butylated hydroxytoluene; BMPF, bis(9-methylphenyl-9-fluorenyl) peroxide; BPA-PC, bisphenol A-based polycarbonate; CBZ, carbamazepine; CE, contact electrification; CNC, cellulose nanocrystals; Crl, crystallinity index; DAAN, diarylacetonitrile; DABBF, diarylbisbenzofuranone; DABBT, diarylbisbenzothiofenonyl; DBU, 1,8-Diazabicyclo[5.4.0]undec-7-ene; DCE, dichloroethane; DIPEA, *N,N*-diisopropylethylamine; DMC, dimethyl carbonate; DMF, *N,N*-Dimethylformamide; DMT, dimethyl terephthalate; DNMA, 4,5-dimethoxy-2-nitrobenzyl methacrylate; DNS, dinitrosalicylic acid; DOTP, dioctyl terephthalate; DP, degree of polymerization; EPO, anthracene endoperoxide; ESR, electron spin resonance; GlcNAc, *N*-acetylglucosamine; G2, Grubbs second-generation catalyst; IBVE, isobutyl vinyl ether; *k*, degradation rate constant; LAG, liquid-assisted grinding; MAPE, maleic anhydride grafted PE; MAPP, maleic anhydride grafted PP; MCC, microcrystalline cellulose; mCPBA, *m*-chloroperoxybenzoic acid; M_{lim} , limiting molecular weight; MMA, methyl methacrylate; M_n , number-average molecular weight; MO, methyl orange; MOF, metal-organic framework; mPEG, α -protected methoxy-polyethylene glycol; M_w , weight-average molecular weight; NEXAFS, near-edge X-ray absorption fine structure; NMP, *N*-methyl pyrrolidone; PAA, poly(acrylic acid); PBMA, poly(butyl methacrylate); PC, polycarbonate; PE, polyethylene; PEEK, polyether ether ketone; PEG, polyethylene glycol; PEO, polyethylene oxide; PEP, poly(ethylene-*alt*-propylene); PET, polyethylene terephthalate; PH, poly(1-hexene); PHPMA, poly[*N*-(2-hydroxypropyl)methacrylamide]; PI, polyisoprene; PLA, polylactide; PMA, poly(methyl acrylate); PMI, process mass intensity; PMMA, poly(methyl methacrylate); PMS, poly(α -methyl styrene); PNB, polynorbornene; POLAG, polymer-assisted grinding; PP, polypropylene; PPM, post-polymerization modification; PPS, polyphenylene sulfide; PS, polystyrene; PTFE, poly(tetrafluoroethylene); PTHF, polytetrahydrofuran; PVA, polyvinyl acetate; PVBA, poly(vinyl benzaldehyde); PVBI, polyvinyl benzyl imine; PVC, polyvinyl chloride; PVDF, polyvinylidene fluoride; PVP, polyvinylpyrrolidone; PV(OH), polyvinyl alcohol; P(Am), polyether with ammonium-based side chains; RAging, reactive aging; RI, refractive index; rt, room temperature; SEC, size-exclusion chromatography; SEM, scanning electron microscope; SET, single electron transfer; SMFS, single-molecule force spectroscopy; SPS, syndiotactic PS; STXM, scanning transmission x-ray microscopy; T_c , ceiling temperature; TASN, tetraarylsuccinonitrile; TCNE, tetracyanoethylene; T_g , glass transition temperature; thp, theophylline; TPA, terephthalic acid; TsCl, 4-toluenesulfonyl chloride; WAXD, Wide-angle x-ray diffraction; WSP, water-soluble products; XRD, x-ray diffraction; D , molecular weight dispersity; δ_c , limiting particle size; 12PGC, Twelve Principles of Green Chemistry; 2-pyr, 2-pyrrolidinone.

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Influence of Crystallinity on the Mechanochemical Degradation of Poly(lactide) with Ball-Mill Grinding

Somin Cha, Jeung Gon Kim,* and Gregory I. Peterson*



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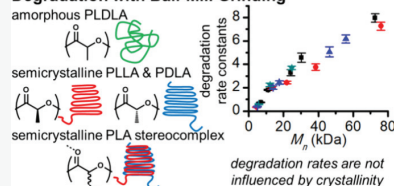
Article Recommendations



Supporting Information

ABSTRACT: Polymers can undergo mechanochemical chain scission during ball-mill grinding (BMG). Various milling and polymer parameters were known to influence chain scission, but the influence of crystallinity was not fully explored. In this report, using ring-opening polymerization, we prepared a library of poly(lactide)s (PLAs) with varying molecular weight and varying crystallinity by changing the stereochemistry of monomers. Semicrystalline PLLA and PDLA (from L- and D-lactide, respectively), amorphous PLDLA (from a copolymerization of L- and D-lactide), and a stereocomplex between PLLA and PDLA were subjected to degradation experiments to assess their degradation kinetics. We found degradation rate constant trends that suggested that the crystallinity had a negligible influence on degradation rates. We attributed this behavior to the fast amorphization of the semicrystalline PLAs. This work provides important insight into how other polymer transformations that occur during ball milling can influence chain scission.

Degradation with Ball-Mill Grinding



INTRODUCTION

Ball-mill grinding (BMG) is an increasingly popular technique for conducting mechanochemical reactions with polymers.^{1–3} Many polymers undergo homolytic chain scission when subjected to ball milling. The reactivity of the resulting macroradicals can be exploited for various applications, such as generating fluorescent species,^{4–7} synthesizing polymers with different composition or topology (e.g., block copolymers and polymer brushes),^{8–11} or depolymerization to monomer.^{12–15} With the incorporation and activation of mechanophores (force-responsive moieties) embedded in the polymer, other functional responses can be achieved, such as mechanochromism,^{16–18} small molecule release,^{19,20} and gated degradability.^{21–23} For applications using mechanophores, homolytic chain scission represents a competitive side reaction that limits the functional response. Given the importance of chain scission, significant effort has been devoted to understanding how various ball milling and polymer parameters can influence it, but important questions still remain.

One of the most important polymer parameters that influences chain scission is molecular weight. Chain scission generally only occurs in polymers that exceed a limiting molecular weight (M_{lim}). Above the M_{lim} , degradation rate constants tend to increase with increasing molecular weight. To provide more insight into this relationship, we recently conducted a systematic study of the influence of molecular weight on the degradation kinetics of amorphous polymers with vibratory ball milling.²⁴ We found that for polystyrene and poly(methyl methacrylate), above 75 to 100 kDa, the degradation rate no longer increased with increasing molecular weight, and instead remained relatively constant. We found a linear relationship between degradation rate constants and the

initial glass transition temperature (T_g) of the polymers, which potentially explained why the degradation rates stopped increasing (the T_g stopped increasing, as described by the Flory–Fox equation).²⁵ While this behavior had been observed in several cases,^{6,24,26,27} it remained unclear if the relationship would also hold true for semicrystalline polymers.

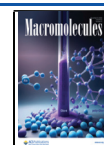
Semicrystalline polymers, such as polyethylene terephthalate (PET),^{28,29} polyethylene,³⁰ and cellulose,³¹ have been shown to undergo chain scission upon ball milling, although molecular weight studies with semicrystalline polymers are rare. In one informative study, Wu, Guo, and co-workers showed that the degradation rate of hydroxyethyl cellulose (HEC) increased with increasing molecular weight within the range of 100 to 250 kDa (viscosity-average molecular weights),³² however, rate constant trends were not further elucidated. Regardless, each polymer's molecular weight decreased and leveled off near the M_{lim} (ca. 6 kDa) after ca. 16 h of vibratory milling. They also showed with wide-angle X-ray diffractometry, that HEC was almost completely amorphized after 8 h of milling. Amorphization is a common phenomenon that has been reported for various polysaccharides and for synthetic polymers such as polyvinyl chloride and PET.^{33,29}

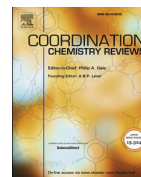
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Review

Emerging porous solids for electrocatalytic and photocatalytic seawater splitting

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ABSTRACT

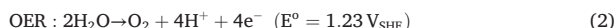
Direct splitting of the seawater by renewable energy sources is pivotal to the practical production of hydrogen because of the reduced energy and cost of water purification. However, formidable challenges remain for realizing the direct utilization of seawater for hydrogen production due to the slow water splitting reaction kinetics under the near-neutral condition (approximately pH 8.0) and the occurrence of side reactions originating from the impurities in the seawater. Design concepts of the catalysts compatible with direct seawater splitting are thus greatly needed to satisfy both catalytic activity and selectivity, and porous solid materials such as metal–organic frameworks and covalent organic frameworks have emerged as a new class of electrocatalysts or photocatalysts toward the direct seawater splitting. Herein, we review the current status of the porous solid materials as new catalyst materials by placing emphasis on the structural features that make these materials particularly attractive for seawater splitting: Non-pyrolyzed porous solid structures, which preserve their inherent porosity and properties of organic linkers, have shown great promises in particular. We also point to the existing limitations and future research directions for placing porous solid-based electrocatalysts and photocatalysts on the horizon of a sustainable energy future.

1. Introduction

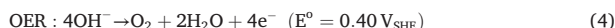
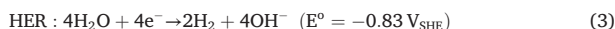
Driven by the urgent requirements to address climate change, extensive research thrusts have been placed on the production and usage of hydrogen as the alternative to conventional carbon-based energy sources [1–3]. However, more than 95 % of hydrogen production currently relies on the by-product hydrogen from fossil fuels, posing considerable challenges [4]. In this context, attempts to produce hydrogen using renewable energy sources during the last few decades place water splitting-driven hydrogen production most promising and sustainable [5,6]. In particular, solar energy has the highest potential to produce hydrogen by concentrated photovoltaic and water electrolysis systems or photocatalytic water splitting [6–9].

Catalytically active materials for the water splitting carry out two crucial redox reactions (Eqs. (1)–(4)): cathodic hydrogen evolution reaction (HER) and anodic oxygen evolution reaction (OER), where SHE represents standard hydrogen electrode [5,10].

Acidic electrolyte;



Alkaline electrolyte;



Because current water splitting technologies are compatible with only highly pure water feeds, most of the catalysts were evaluated under pure distilled water or specific acidic (H_2SO_4 , HClO_4 , etc.) or basic (KOH , NaOH , etc.) electrolytes [11,12]. However, the demands for larger quantities of freshwater feedstock pose a daunting challenge in places where the supplement of freshwater is limited [13]. Accordingly, research efforts have been directed to develop catalysts for direct seawater splitting, while several challenges linked to the side reactions induced by impurities in seawater still remain [14–17]. Securing the HER- and OER-selectivity and suppressing unwanted side reactions have

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Ultrahigh Electrode Performance of Low-Loaded Iridium Jagged Nanotubes for Water Electrolysis Applications

Jun Kim, Taehyun Kwon, Jaeseung Lee, Hyun Ju Lee, Minki Jun, Hyung Chul Ham, Hyunchul Ju, Sangwon Kim, and Jin Young Kim*

Highly active Ir electrocatalysts based on ultralow loading for proton exchange membrane water electrolysis (PEMWE) applications are desirable to achieve gigawatt production levels. However, no clear direction has been developed yet to achieve that scale. Here, newly-synthesized Ir-based jagged nanotubes (J-IrNTs) with the ultrahigh electrode performance of $10 \text{ A mg}_{\text{Ir}}^{-1}$ at 1.6 V in a PEMWE cell at Ir loadings as low as $0.05 \text{ mg}_{\text{Ir}} \text{ cm}^{-2}$ are reported, outperforming previously reported Ir-based electrocatalysts. The J-IrNT also exhibits only marginal degradation in stability tests. Notably, the resulting electrode of J-IrNT provides enhanced intrinsic catalytic activity and high surface area for efficient electrocatalysis, with hierarchical branching composed of interconnected multiscale channels and highly corrugated surface textures, for facile mass transport. The findings demonstrate that the jagged nanotubular structure is a promising approach for overcoming the limitations associated with Ir-based electrocatalysts and developing sustainable advanced PEMWE.

membrane water electrolysis (PEMWE) holds immense potential for the production of green hydrogen. It can be efficiently coupled to intermittent renewable energy sources, making it one of the key candidates among prospective sustainable energy systems.^[5,6]

Compared to the other electrolysis technologies, large-scale PEMWE development offers several advantages, including a large current density, high hydrogen purity, and compact construction. However, the cost of producing green hydrogen remains a significant barrier. Of particular concern is the high loadings of iridium (Ir) electrocatalysts in the anode, typically $1\text{--}3 \text{ mg}_{\text{Ir}} \text{ cm}^{-2}$, for the oxygen evolution reaction (OER), a requirement imposed by the corrosive electrochemical conditions inherent to the PEMWE.^[7,8] To enable the large-scale

deployment of PEMWE at gigawatt scale, it will be necessary to significantly reduce the Ir loading, ideally reaching levels as low as $0.05 \text{ mg}_{\text{Ir}} \text{ cm}^{-2}$.^[9–11]

Intensive attention has been directed to developing novel and efficient Ir-based electrocatalysts to improve catalytic activity without sacrificing durability. At the catalyst level, advanced nanocatalysts have shown promising results with high efficiency using less Ir,^[12,13] but their practical application in PEMWE cells

1. Introduction

To address global climate change and the prevailing energy crisis that has plagued our world in recent years, there has been an unprecedented surge in research to develop sustainable and environment-friendly energy resources. Among the array of potential solutions, green hydrogen stands out as a particularly promising contender.^[1–4] In particular, proton exchange

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REVIEW

Exploration of metal-free 2D electrocatalysts toward the oxygen electroreduction

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Abstract

The advancement of economical and readily available electrocatalysts for the oxygen reduction reaction (ORR) holds paramount importance in the advancement of fuel cells and metal-air batteries. Recently, 2D non-metallic materials have obtained substantial attention as viable alternatives for ORR catalysts due to their manifold advantages, encompassing low cost, ample availability, substantial surface-to-volume ratio, high conductivity, exceptional durability, and competitive activity. The augmented ORR performances observed in metal-free 2D materials typically arise from heteroatom doping, defects, or the formation of heterostructures. Here, the authors delve into the realm of electrocatalysts for the ORR, pivoting around metal-free 2D materials. Initially, the merits of metal-free 2D materials are explored and the reaction mechanism of the ORR is dissected. Subsequently, a comprehensive survey of diverse metal-free 2D materials is presented, tracing their evolutionary journey from fundamental concepts to pragmatic applications in the context of ORR. Substantial importance is given on the exploration of various strategies for enhancing metal-free 2D materials and assessing their impact on inherent material performance, including electronic properties. Finally, the challenges and future prospects that lie ahead for metal-free 2D materials are underscored, as they aspire to serve as efficient ORR electrocatalysts.

KEYWORDS

2D materials, electrocatalysts, non-metal, oxygen reduction reaction

1 | INTRODUCTION

The exploration of green and renewable energy generation systems such as fuel cells and metal-air batteries has become a necessity in recent years due to the rapid growing issues such as climate change, environmental pollution, and energy security.^[1–7] To promote the performance of these energy systems, it is crucial to understand the fundamental principles of electrocatalysis.^[8–15] Oxygen reduction reaction (ORR) is an electrochemical process in which O₂ is reduced by four electrons at the cathode of fuel cells and metal-air batteries,^[16–19] and the sluggish ORR kinetics that limits the overall cell performance has been known as a major problem.^[20–23]

To tackle this issue, high-performance Pt-based catalysts have been adopted,^[24–29] but their scarcity in the earth, high cost, and low stability restrict the large-scale application.^[10,30,31] As an alternative, remarkable advances have been achieved in the development of non-noble metal-based catalysts, and some of them have demonstrated ORR activity similar to or even superior to the benchmark Pt catalyst.^[32–37] However, non-noble metal-based catalysts are much less stable under high operating potentials during ORR. Apart from the development of non-noble metal-based catalysts, the recent discovery of metal-free carbon-based ORR catalysts has opened up new horizons.^[38–42] Metal-free carbon-based catalysts boast advantages in tolerance to the fuel, stable chemical structure, and good stability in alkaline media.^[43–45]

Joyjit Kundu and Taehyun Kwon contributed equally to this work.

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Enhancement of Optical Gain in Colloidal CdSe/CdS/ZnS Quantum Dots through Nanosecond Optical Pumping

Suhyeon Kim, Suhyeon Hwang, and Jiwon Bang*



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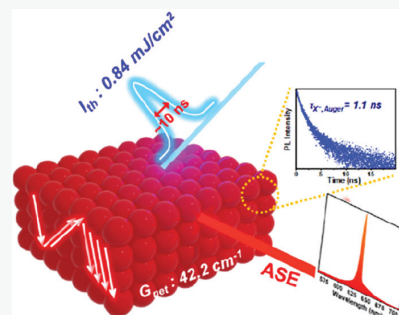


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Supporting Information

ABSTRACT: Optical gain and lasing in colloidal nanocrystals are often hindered by sub-nanosecond rapid Auger non-radiative recombination, especially under continuous wave optical or electrical excitation. This study demonstrates amplified spontaneous emission (ASE) from CdSe/CdS/ZnS quantum dot (QD) solids through prolonged pulsed optical pumping over 10 ns. The incorporation of CdS and ZnS double shells on CdSe QDs effectively decelerates the Auger process in multiexcitonic states by extending the electron wave function and enhancing dielectric screening. Furthermore, we engineer smooth, densely packed QD solid films that efficiently guide the optical mode, achieving substantial net gain values under nanosecond pumping. The proposed approach helps observe ASE with gain thresholds of 0.84 and 1.5 mJ/cm² under optical pumping pulse widths of 6 and 15 ns, respectively. This advancement can promote continuous pumping in colloidal QD gain systems, opening new avenues for optoelectronic applications.



In the pursuit of novel active materials for photonic applications, colloidal quantum dots (QDs) have garnered extensive attention owing to their exceptional optical performance. Precise control of the composition and size of these nanomaterials facilitates spectral tunability with narrow bandwidths. In particular, colloidal QDs can attain near-unity photoluminescence (PL) quantum yields (QYs) and exhibit robust stability under strenuous conditions, which can be attributed to the presence of inorganic protective shell layers. Furthermore, the solution processability of colloidal QDs renders them compatible with a wide range of substrates, ensuring scalability and promoting their integration into various photonic systems.^{1,2}

Colloidal QDs based on cadmium selenide (CdSe), known for their bright emission in the visible range and high photochemical stability, have been the focus of extensive research.¹ Their potential as optical gain media in amplified spontaneous emission (ASE) and lasing systems has attracted significant research interest. In the single exciton state of CdSe QDs, stimulated emission is compensated by light absorption from the remaining valence electron in their 2-fold degenerate band-edge states, leading to optical transparency. Thus, to demonstrate ASE, population inversion in the biexciton state of CdSe-based QDs is necessary to enable light amplification by eliminating ground state absorption.^{3,4} However, the biexciton state in QDs introduces a rapid non-radiative Auger recombination pathway that directly competes with stimulation emission. Notably, the Auger recombination rates in strongly confined semiconductor nanocrystals are several orders of magnitude higher than those of radiative decay, significantly impeding the optical gain of colloidal QDs.^{3–5}

Since the initial demonstration of the optical gain properties of colloidal QDs,⁶ significant research over the past 2 decades has focused on enhancing their optical gain and lasing properties under optical pumping.^{3,4,7} Various researchers have attempted to reduce the gain threshold by slowing Auger non-radiative recombination^{8,9} or suppressing ground state absorption.^{10,11} However, the realization of practical QD-based ASE devices remains a substantial challenge. Most of the existing strategies adopt ultrashort, femtosecond optical pumping to establish the population inversion state of QD gain media without interference from Auger-type relaxation during excitation.³ In such scenarios, the Auger recombination process considers only biexciton relaxation, with competition for stimulated emission. Such ultrashort pulse pumping conditions restrict the practicality and cost-effectiveness of QD-based lasers. Moreover, when QDs are pumped with pseudo-continuous optical waves or direct current electrical injection, the rapid Auger process impedes the establishment of the population inversion state in the gain media, further suppressing ASE.

To address these limitations, this work was aimed at demonstrating optical gain in densely packed CdSe/CdS/ZnS (core/shell/shell, C/S/S) QDs under nanosecond optical

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Cluster-mediated synthesis of monodispersed In(Zn)P quantum dots and their ZnSe/ZnS shell growth for bright and narrow band emission

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Photoluminescence

ABSTRACT

Colloidal quantum dots (QDs) have gained significant attention as potential light emitters for wide-color-gamut displays owing to their remarkable photophysical properties. Among them, zinc-incorporated InP-based (In(Zn)P) QDs are particularly promising for real-life display and lighting applications because they are heavy-metal-free, and exhibit excellent brightness. However, achieving a narrow emission linewidth, which is crucial for high color purity in displays, as well as ensuring reproducibility between batches, remains a challenge due to the uncontrollable nucleation and subsequent growth kinetics of these QDs. In this study, we investigate the shortcomings of conventional heating method adopted for preparing In(Zn)P QDs, which results in poor size distribution and batch-to-batch variation. Furthermore, stable Zn-P molecular complex intermediates in the reaction mixture hinder concurrent nucleation and size-focusing growth, leading to undesired variations in QD formation. Therefore, we propose a novel heat-up-based synthesis protocol for In(Zn)P QDs by reacting metastable pre-nucleated InP clusters with added Zn atoms while elevating the temperature. The preformed InP cluster acts as a continuous monomer reservoir, leading to the size-focused growth of In(Zn)P QDs, and significantly improving the photoluminescence (PL) bandwidth and batch reproducibility. Moreover, by carefully optimizing the coating conditions with a ZnSe/ZnS double shell, we achieve brighter PL with monoexponential decay and a narrower PL linewidth for green-light-emitting In(Zn)P/ZnSe/ZnS QDs. By addressing the challenges associated with size distribution and batch reproducibility, our findings contribute to the development of high-performance QD-based displays with improved color quality.

1. Introduction

Colloidal quantum dots (QDs) possess remarkable photophysical properties, such as bright and narrow photoluminescence (PL) emission, tunable PL energies, and robustness to photochemical stresses, which make them ideal light emitters for wide-color-gamut displays [1,2]. Cd (S,Se)-based QDs and Pb-halide perovskite QDs have been extensively studied as photon sources in the visible-color range [2,3], but their intrinsic toxicity limits their commercial application. Alternatively, heavy metal-free InP QDs have been recognized as highly promising emissive materials for real-life displays and lighting applications because they cover the entire visible spectral range by modulating their confined potentials. The brightness of InP-based QDs has been significantly improved through various efforts, such as the modulation of shell composition and their surface, which can lead to a near-unity PL quantum yield (QY) [4–6].

Conversely, for obtaining high color purity displays, the narrow spectral width of emissions is a critical issue [4]. The ensembled PL emission lines of QDs are mainly governed by their size distribution. The evolution of II-VI QDs follows the Lamer model, starting with homogeneous nucleation, followed by size-focused growth achieved by the appropriate combination of cation and anion precursors, resulting in the formation of uniform colloidal particles [7,8]. However, the colloidal synthesis of InP QDs poses a challenge while regulating the nucleation and growth processes to achieve a monodisperse size distribution. These limitations are attributed to the highly reactive nature of the group-V organometallic precursors, such as trimethylsilyl-based compounds. The rapid depletion of the precursor in the initial state of InP QD formation leads to ripening-dominated growth rather than size-focused growth from the monomers. Furthermore, the more covalent nature of the III-V InP bond compared to the more ionic bonding character of II-VI and IV-VI compound semiconductor QDs, such as CdSe and PbSe, also

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Colour-Passive radiative cooling in optoelectronics with Silver/Quantum dot decorated silica multifunctional hybrid structures

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ABSTRACT

The integration of solar cells into the architectural design is hindered by challenges such as passive cooling and colouration appeal. The present study proposes an approach to overcome these obstacles by developing colourful solar cells that maintain optimal performance while addressing limitations related to heat accumulation from plasmon metal nanoparticles and quantum dots (QDs). To attain this, silver/silica microsphere composites combined with CdSe/ZnS core/shell QDs are fabricated with two distinct sizes of Ag nanoparticles embedded around SiO₂ microspheres. Upon coating CIGS cells with this hybrid structure, a visually striking vivid green colouration is observed, while ensuring performance efficiency. Furthermore, the radiative cooling capability of these cells is evaluated through indoor and outdoor experiments. Significantly, a substantial temperature reduction of up to 3.2 °C in CIGS cells is achieved when the hybrid structures were applied to the cell's surface under high solar irradiance. The study represents a significant contribution to innovative strategies targeted at achieving passive cooling effect and enhancing aesthetic integration in building-integrated photovoltaics applications.

1. Introduction

Building integrated photovoltaics (BIPVs) are gaining traction in modern construction due to their efficiency and easy installation, with predictions suggesting an annual growth rate of 25 % by 2030 [1]. Their incorporation in vehicles has also led to a 63 % reduction in greenhouse gas emissions [2]. However, the attempt to increase the power output of PV systems leads to predominantly black modules for optimised light absorption, which limits their aesthetic appeal [3,4]. Recent PV advancements have started focusing on enhancing the visual appearance of modules, offering varied colours for better integration into unique architectural designs and addressing potential health concerns for the human eye [5–7]. The colour of a solar cell results from visible light being either reflected or transmitted through it, representing an optical loss in photovoltaic solar energy conversion [8,9]. Thus, ensuring the preservation of solar cell efficiency while incorporating colour is crucial for effective implementation.

Highly efficient photoluminescence (PL) materials, such as quantum

dots (QDs), whose emission properties intrinsically correlate with their colour-tune ability, have been intensively attracted in recent years [10–15]. Specifically, when the diameter spans from 2 to 10 nm, the emitted light transitions from blue to red wavelengths. Among them, QDs-based cadmium chalcogenides (CdSe, CdS, CdTe) and their core/shell configurations stand out as one of the most promising materials for producing colour-tuned solar cells [16]. These QDs exhibit exceptional optical characteristics essential for optoelectronic devices, including tunable bandgap, high quantum yield, excellent photostability, absorption spectrum across a wide spectral range and intense luminescence in both the ultraviolet (UV) and visible regions [17,18]. Besides, the PL emission of QDs can significantly impact the efficiency of solar cells by improving light trapping, and enabling the generation of multiple electron-hole pairs from a single photon, thereby maximizing the utilization of incoming solar radiation for electricity generation [13,19]. This highlights the dual functionality of QDs, serving as the chromatic appearance while optimistically impacting solar cell performance [20].

Nevertheless, the relatively low absorption coefficient and the

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ARTICLE

Organometallic ruthenium complexes derived from anthracene and pyrene chromophores: Synthesis and photophysical properties

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Abstract

Two new monopyridyl-based Anthracene and Pyrene ligands were synthesized and employed to design Ruthenium-coordinated organometallic complexes, **RuAntra** and **RuPyrene**, respectively. The ligands and their metal complexes were fully characterized by different analytical techniques, including multinuclear 1D- and 2D-NMR, electrospray ionization-mass spectrometry, UV-Vis, fluorescence spectroscopy, and elemental analysis. Furthermore, their photophysical properties were studied in different solvents with increasing polarity using UV-Vis and fluorescence spectroscopy, showing less or no significant solvolysis effect. The average emission lifetimes of the complexes were further determined using time-resolved emission spectroscopy. Additionally, the ligands and their metal complexes showed excellent ability to generate singlet oxygen and can be useful for several important potential applications.

KEYWORDS

anthracene, organometallic complexes, photophysical properties, pyrene, ruthenium

In organometallic chemistry, ruthenium-arene-based complexes have been developed as a promising field of research due to their remarkable applications in various fields of chemistry and biology.^{1,2} Due to their straightforward synthesis using easily accessible arene metal precursors and organic ligands, these complexes are being considered promising candidates for use as catalysts in a variety of organic chemical transformations and biological applications, including the development of several water-soluble anticancer drug candidates.^{3,4}

The synthesis of ruthenium metal complexes typically involves the reaction of ruthenium metal precursors with several pyridine, bipyridine, or terpyridine derivative ligands to form diverse coordinated metal complexes.⁵ The choice of ligand, metal source, and reaction conditions can influence the structure and overall properties of the resulting metal complexes.⁶ Dinuclear ruthenium clips, when treated with bidentate, tridentate, or tetradentate pyridine ligands, can produce rectangle, prism, or cage-type

metal macrocycles, respectively.⁷ These ruthenium complexes with pyridine-based ligands find diverse applications across several fields, including catalysis, medicine, host-guest chemistry, material science, and photocatalysis.⁸

In particular, metal-based complexes based on pyrene and anthracene chromophores exhibit diverse applications in optoelectronics, catalysis, and sensors.⁹ Due to their superior photoluminescence properties and high chemical stabilities, metal complexes based on these chromophores are good candidates for developing fluorescence sensors.¹⁰ An appropriate selection of donor-acceptor pairing in a metal-organic system can produce versatile photophysical properties, such as charge and energy transfer and exciplex or excimer emission.¹¹ The photophysical properties of such systems can also be fine-tuned using solvents of different polarities or mixed solvents that generate aggregation-based emission behaviors.^{10a,12} Herein, we synthesized two new pyrene and

Narrowing the photoluminescence bandwidth of InP-based colloidal quantum dots through photon-triggered isolation

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Abstract

The optimization of photoluminescence (PL) spectral bandwidths in InP-based colloidal quantum dots (QDs) faces limitations with traditional synthetic methods. This study introduces a novel approach utilizing light-induced thiolate ligand detachment to selectively precipitate QDs within an ensemble, triggered by photons whose energy matches the red tail of the band-edge absorption peak. We demonstrate that incorporating benzoquinone as an electron acceptor under inert conditions crucially enhances the efficiency of photo-excited hole-induced ligand detachment. Through careful optimization of laser exposure conditions, we successfully narrowed the PL bandwidth of InP/ZnSe/ZnS QDs from 39 to 35 nm. Our findings offer significant insights into the development of high-performance QD-based displays, promising advancements in color purity.

KEYWORDS

bandwidth, colloidal quantum dot, ligand, photoluminescence, photo-oxidation

Fluorescent materials capable of emitting pure color have potential applications in high-color purity display technology and multiplexing biosensors. In this context, inorganic semiconducting colloidal nanoparticles, known as quantum dots (QDs), have attracted intense interest for their narrow emission bandwidth.¹ This characteristic is distinctively more defined than that of organic or polymer fluorophores, which typically exhibit a broader emission bandwidth due to vibronic coupling during the relaxation of the excited state.

The size-dependent optical band gap tunability of QDs enables adjustable photoluminescence (PL) peaks. However, this tunability often leads to the PL broadening caused by size inhomogeneity in ensembles.² Pyrolysis-based synthesis of colloidal II–VI QDs, such as CdSe, has dramatically improved over recent decades,³ resulting in the formation of uniform colloidal particles and a narrowing PL bandwidth to levels nearly identical to those at single-dot level.⁴ However, the intrinsic toxicity of Cd-containing QDs limits their commercial application. As an alternative, heavy metal-free indium phosphide (InP) nanocrystals have emerged as promising emissive materials for real-life displays and sensing applications because they cover the entire visible spectral range by

modulating their sizes.⁵ However, the colloidal synthesis of InP QDs is challenging, particularly in controlling nucleation and growth processes to achieve a narrow size distribution.^{5a,6} Despite numerous efforts to enhance size uniformity, achieving ensemble emission colors of uniformity in InP-based QDs has been a difficult challenge, with the PL bandwidth only recently narrowing to less than 35 nm.^{5c} However, size inhomogeneity remains an issue, and developing synthetic protocols to obtain a nearly monodispersed PL line shape, a feat already accomplished with CdSe QDs, continues to be a significant hurdle.⁷

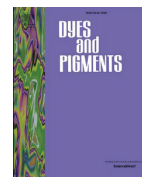
Post-treatment techniques such as size selection purification process based on density gradient centrifugation can effectively isolate similar-sized QDs from ensemble.^{2a,8} However, the purification of bare InP QDs, primarily related to the optical band gap of InP-based type-I core/shell QDs, induces surface oxidation, resulting in a blueshift and broadening of their optical band gap.⁹ On the other hand, the optical band gap of InP QDs decorated with shell layers does not directly correlate with the diameter of core/shell QDs. Thus, the size selection of core/shell QDs does not straightforwardly lead to isolation based on optical band gap variations. Consequently, the size-selective purification has not been efficient for both



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Fabrication of ZnO-quantum dot hybrid nanocomposites with enhanced light scattering and emission characteristics for micro LED display applications

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ABSTRACT

Herein, a facile method is reported to fabricate a color conversion layer comprised of zinc oxide nanoparticles (ZnO NPs) and quantum dots (QDs) for micro-light emitting diode (μ -LED) displays. Further, 11-mercaptopentanoic acid (MUA)-treated ZnO NPs (ZnO@MUA NPs) are synthesized via a high-energy ball milling method, which bypasses the need for complex synthesis processes and enables functional groups to attach to the surface, thereby facilitating the formation of ZnO@MUA NPs-QD hybrid composites (ZQCs). The particle size, surface characteristics, elemental composition, and binding energy are analyzed to elucidate the reaction mechanism and to confirm that the fabricated ZQC exhibits a chemically bonded structure. To ascertain the optimal molar concentration for incorporating the QDs, parameters such as QD bonding efficiency, quantity of bonded QDs, interparticle distance, and absolute photoluminescence quantum yield (PLQY) are evaluated as functions of molar concentration. As a result, the optimal molar concentration is found to be 1930 nM, giving a bonding efficiency of 96.8 %, along with the bonded QDs of 1.51×10^{11} per a unit area of 1 mm^2 of ZnO NPs and a PLQY of 64.8 %. Furthermore, comprehensive optical analyses are performed to assess the potential of the ZQC as a color conversion material for μ -LED displays, in comparison to the use of pristine QDs and silica NP-QD composites.

1. Introduction

Light-emitting diodes (LEDs) that have been miniaturized to less than $100 \text{ }\mu\text{m}$ in size while retaining the functionality of the traditional LEDs used for lighting are termed micro-LED (μ -LEDs). The first high-resolution display based on indium gallium nitride μ -LEDs in video graphics array (VGA) format was unveiled in 2011 [1,2]. Technologies associated with μ -LEDs are emerging as the next generation of display technology due to advantages such as high contrast ratio, response speed, color gamut, viewing angle, brightness, and longevity. Applications such as wearable displays, autonomous vehicle sensors, healthcare, internet of things (IoT) communications, and miniature robots are intricately associated with μ -LED technology [3,4]. Nevertheless,

several challenges, especially the realization of full-color displays, need to be addressed before μ -LED displays can be commercialized.

The two general approaches to using μ -LEDs as display pixels are as follows: (i) using individual red, green, and blue (RGB) μ -LEDs as sub-pixels, and (ii) applying color filters based on color conversion materials (CCMs) on ultraviolet (UV) and blue LEDs as a backlight source [4–6]. In the first approach, each chip is transferred and positioned on a substrate to form pixels. This manufacturing process is notably complex and costly. Moreover, due to the varying luminous efficiencies and degradation rates of RGB μ -LEDs, sophisticated drive circuits are essential for maintaining the color rendering index during operation [4, 6]. In the second approach, however, full-color rendering is achieved without a complex mass transfer process by placing CCMs on UV/blue

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Heterostructure seed-mediated synthesis of zinc phosphide quantum dots for bright band-edge emission†

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This study explores the synthesis of colloidal zinc phosphide quantum dots (QDs) by a novel In(Zn)P cluster seed-mediated approach, addressing the challenge of achieving low-cost, high-quality, nontoxic QDs suitable for optoelectronic applications. By intentionally limiting the amount of In precursor added to a hot solvent containing Zn and P precursors, In-rich In(Zn)P cluster seeds were formed. Subsequently, these clusters served as seeds for the growth of zinc phosphide nanocrystals, effectively using the remaining Zn and P precursors for further crystal growth. The synthesized QDs exhibited a tetragonal-like Zn₃P₂ structure and exceptional optical properties, including band-edge photoluminescence (PL) emission under ambient conditions. A ZnS shell was applied to further enhance the PL intensity, achieving a PL quantum yield of 40% and an average PL decay lifetime of 74 ns, while significantly improving the stability of the QDs. Temperature-dependent PL spectroscopy revealed significant resistance to thermal quenching with an exciton dissociation energy of 62 meV, underscoring the potential of this approach for advancing the field of optoelectronics. This method provides a pathway to fabricate zinc phosphide-based QDs with controlled optical properties and highlights the effective use of earth-abundant materials in the development of environmentally benign photonic materials.

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Introduction

Colloidal quantum dots (QDs) have garnered considerable attention in the field of optoelectronics owing to their unique optical properties, which can be tuned with the size, shape and composition of the QDs.¹ Although Cd-chalcogenide and Pb-halide perovskite QDs have excellent optical properties in the visible range, their toxicity restricts their practical applications. As an alternative, heavy-metal-free InP-based QDs have been extensively studied owing to their excellent light emission properties, including intense and stable emissions with narrow bandwidths in the visible spectral range. These QDs have already been integrated as down-converting phosphors in displays and are being considered for other applications, such as biomedical imaging and sensors.^{2–5} However, the scarcity and high cost of In pose challenges to the widespread use of InP-based QDs, driving the search for more sustainable alternatives.

Binary zinc phosphide crystals exist in various compounds such as Zn₃P₂, ZnP₂, and ZnP₄, with tetragonal-Zn₃P₂ being

the most stable phase under zinc-rich conditions among the various Zn–P compounds with polymorphs.^{6,7} Tetragonal-Zn₃P₂, an earth-abundant semiconductor characterized by a large optical absorption coefficient,⁸ an ideal direct bandgap of 1.5 eV suited for solar light harvesting, and a long minority carrier diffusion length (up to 10 μm),^{9,10} which enables effective responses to visible and near-infrared light. Thus, Zn₃P₂ has emerged as a promising candidate for optoelectronic applications, particularly as a photovoltaic material.¹¹ In addition to the light-absorbing capabilities of Zn₃P₂, quantum-confined Zn₃P₂ nanoparticles with controlled sizes can emit across the visible spectral range. However, the synthesis of nanosized colloidal Zn₃P₂ QDs is associated with considerable challenges. The process requires burst nucleation and subsequent crystal growth under high-temperature pyrolysis, which is critical for controlling the QD size and enhancing its optical properties.^{12,13} Furthermore, the limited availability of suitable precursors and challenges in managing the colloidal synthesis process often result in QDs with suboptimal excitonic absorption and emission properties.

Dialkyl zinc, the commonly used Zn source for preparing Zn₃P₂ QDs via the organometallic route,^{11,14–16} is highly pyrophoric, explosive, and costly. This highly reactive zinc precursor is difficult to control, does not produce reproducible results, and is prone to rapid deactivation via oxidation.¹⁷ Alternatively, zinc carboxylates have been studied as precur-

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journal homepage: www.elsevier.com/locate/jiecEnhancement of photocatalytic NO oxidation by Ag-sulfide on TiO₂ mixed with Na-ZSM-5

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ABSTRACT

Enhancing NO oxidation performance is pivotal for integrating photocatalysts with wet scrubbers in continuous flow systems. Despite the recent development of various catalysts, such as MOFs, our goal was to simplify the composition by using photocatalyst + zeolite catalyst, bringing us closer to a catalyst that can be practically applied. In this study, we have utilized Ag₂S doping and Na-ZSM-5 mixing to improve the photocatalytic NO oxidation performance. Results showed that Ag₂S doping improved visible light absorbance and NO oxidation, despite the observed fluctuations in NO conversion being attributable to mass transfer limitations. Mixing with Na-ZSM-5 compensated for the mass transfer limitations and improved its average NO conversion from 19 to 34%. The Na-ZSM-5 increased the NO adsorption capacity considerably to 0.67 mmol g⁻¹, which reduced the fluctuations in the catalytic performance; subsequently, the diffusion of NO led to its oxidation. Detailed analyses using NO-TPD and DRIFTS further elucidated these improvements, highlighting the significance of optimizing both oxidation reaction and mass transfer rates in the photocatalytic process. We believed that the composite of Ag₂S-TiO₂ + Na-ZSM-5 could simplify the ways to improve the photocatalytic NO oxidation performance and make it closer to practical applications.

Introduction

Nitrogen oxides (NO_x) are known for their harmful effects on both the environment and human health, leading to issues such as acid rain and photochemical smog, while also contributing to the greenhouse effect [1]. NO_x is predominantly generated via high-temperature combustion of chemical fuels, called thermal NO_x, and these NO emissions necessitate effective mitigation strategies [2,3]. Although existing technologies like the three-way catalyst (TWC) and the selective catalytic reduction (SCR) facilitate conversions of NO_x to N₂ [4], they face limitations due to high installation costs, deactivation by sulfur oxide (SO_x) poisoning and their extensive equipment footprint [5–7]. Conversely, wet scrubbers present a promising solution for the

simultaneous removal of SO_x and NO_x, offering a simpler equipment structure that results in a more economic mitigation strategy [8]. To remove NO, the process begins with its oxidation to NO₂, followed by absorption by wet scrubbers. NO₂ is more reactive than NO, making it easier to utilize as well as more soluble in water, so an oxidation process is required from NO to NO₂ [9]. The oxidation process crucially depends on the rapid conversion of NO to NO₂, allowing subsequent NO₂ dissolution in the aqueous phase. Presently, two oxidation methods exist: (1) ozonation and chlorination, as the leading oxidant-based techniques and (2) catalysis [10,11]. Despite the widespread use of oxidant-based technologies, these methods have the potential to release toxic chlorinated or ozonized by-products, spurring extensive research into integrating wet scrubbers with photocatalysts.

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ARTICLE

Enhancing the durability of anion exchange membrane water electrolysis cells via interfacial membrane–ionomer crosslinking

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Abstract

Research into enhancing the performance and durability of anion exchange membrane (AEM)-based water electrolysis (AEMWE) cells has primarily aimed to facilitate commercialization efforts and improve the chemical stability of AEMs, with anion exchange ionomers (AEIs) playing an increasingly important role. This study aimed to reduce ionomer swelling and enhance AEM–catalyst adhesion via AEM–ionomer crosslinking within a membrane electrode assembly (MEA), using allyl- and quaternary ammonium-functionalized poly(phenylene oxide) (PPO), allyl-m-PPO, as both the AEM and AEI. Bis(aryl azide) was added as a crosslinker to an ionomer solution to generate the MEA, which was heated to induce allyl–azide crosslinks at the membrane–ionomer interface. Peel test indicated an increased adhesion at both electrodes for the crosslinked x-allyl-0.05-PPO (5 mol% allyl content relative to the total ion-conducting head group content). Further, x-allyl-0.05-PPO exhibited a smaller voltage increase due to crosslinking compared to TA-PPO, signifying a notable improvement in durability.

KEYWORDS

anion exchange membrane water electrolysis, durability, improved adhesion, interfacial crosslinking, ionomer

INTRODUCTION

Water electrolysis (WE) stands out as a promising and eco-friendly approach for producing carbon-free green hydrogen and achieving a carbon-neutral society, consequently attracting significant research interest in recent years. WE utilizing polymer electrolyte membranes can be categorized as proton exchange membrane water electrolysis (PEMWE) or anion exchange membrane water electrolysis (AEMWE).^{1–3}

Notably, AEMWE holds a distinct advantage over PEMWE as it operates under high pH (alkaline) conditions, eliminating corrosion risks. Therefore, the process allows for the use of nonprecious metal catalysts, such as Co, Ni, and Mo, rather than high-cost precious metal catalysts, unlike PEMWE.^{4,5} Furthermore, AEMWE primarily utilizes hydrocarbon-based polymer electrolyte membranes instead of high-cost perfluorinated membrane materials,

providing a competitive edge in terms of cost-effectiveness.^{6–9} Against this backdrop, AEMWE has been garnering significant research attention.

High electrolytic efficiency and durability are required in the advancement and commercialization of AEMWE cells. In their actual operation, however, the overall performance degrades over time, ultimately affecting the long-term durability of the entire system; various approaches have been proposed to address this limitation.^{5,10–13}

One of the main factors affecting the durability of AEMWE cells is the chemical deterioration of anion exchange membranes (AEMs) by hydroxide ions, reducing their performance, especially at high temperatures.^{5,12–15} In this regard, enhancing the chemical (or alkaline) stability of AEMs and anion exchange ionomers (AEIs), key components of AEMWE cells, can form an effective and practical strategy to improve both cell durability and efficiency.

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Crosslinked high-performance anion exchange membranes based on poly(dibenzyl *N*-methyl piperidine) and pentafluorobenzoyl-substituted SEBS†

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The high ionic conductivity and chemical stability of poly(aryl piperidinium) (PAP) underpin its extensive use in anion exchange membranes (AEMs) for water electrolysis (WE) and fuel cell (FC) applications. Nonetheless, the limited elongation of PAP-based AEMs negatively impacts cell performance and durability. Their high ion exchange capacity (IEC), while enhancing cell performance, often causes excessively high water uptake and diminishes the mechanical properties and phase separation performance. This study developed high-performance AEMs by crosslinking two polymers with contrasting characteristics: poly(dibenzyl *N*-methyl piperidine) (PDB), a rigid PAP-based unit, and poly(styrene-*b*-ethylene-*co*-butylene-*b*-styrene) (SEBS), a flexible unit with good phase separation. Notably, the pentafluorobenzoyl group was incorporated into SEBS to enhance microphase separation. AEMs were developed with pentafluorobenzoyl contents ranging from 4 to 12% (labeled as *x*-PDB-*m*-F5-SEBS, *m* = 4, 8, and 12), and the results were compared with the *x*-PDB-0-F5-SEBS membrane without the pentafluorobenzoyl group. All *x*-PDB-*m*-F5-SEBS membranes exhibit low hydrogen permeability, excellent chemical stability, and a conductivity retention of over 99% after 1080 h. *x*-PDB-4-F5-SEBS demonstrated the most pronounced microphase separation, coupled with high ionic conductivity. It also achieved excellent WE cell performance (3.204 A cm^{−2} at 1.8 V), significantly surpassing that of commercial membranes including FAA-3-50 and PiplerION. Even when using a non-precious metal catalyst (Ni₂Fe) in place of IrO₂, the membrane exhibited a cell performance of 3.364 A cm^{−2} at 1.8 V, equivalent to that achieved with precious metal catalysts. When applied to AEM-based FCs, *x*-PDB-4-F5-SEBS also exhibited a high-power density of 785 mW cm^{−2}, highlighting its significant potential as an AEM in WE and FC applications.

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Introduction

Escalating concerns about climate change and fossil fuel depletion have led to an increased focus on sustainable energy sources. Notably, fuel cells (FCs) use hydrogen fuel (stored as a gas or liquid and exhibiting a high energy density) to generate electricity at a maximum energy conversion efficiency of approximately 80%, and are highly eco-friendly, forming only water as a by-product.^{1,2} Like FC technology, water electrolysis

(WE) to produce hydrogen is environmentally benign because it does not involve carbon dioxide emissions. Thus, this technology has recently garnered significant attention as a key factor in establishing the hydrogen economy.^{3–5}

FC and WE technologies employing polymer membranes as electrolytes are popular research areas due to the relatively low cell operating temperatures involved and their potential for implementation in compact systems. The membranes can be categorized as proton exchange membranes (PEMs), which conduct protons (H⁺), and anion exchange membranes (AEMs), conducting hydroxide ions (OH[−]).

The generally acidic operating conditions of PEM-based FC and WE systems increase the susceptibility of their metallic components (electrocatalysts and bipolar plates) to corrosion. Moreover, the PEM-based FC cathode and WE cell anode both exhibit a high standard electrochemical potential (*E*⁰) of 1.23 V, necessitating the use of noble metal catalysts (e.g., Ru, Pt, and Ir).^{6,7} In contrast, the basic conditions of AEM-based FC and WE

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Enhancing CO₂ Transport Across the PEG/PPG-Based Crosslinked Rubbery Polymer Membranes with a Sterically Bulky Carbazole-Based ROMP Comonomer

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A series of poly(ethylene glycol)-*block*-poly(propylene glycol) (PEG/PPG)- and 5,6-di(9H-carbazol-9-yl)isoindoline-1,3-dione (2CZPIImide)-based crosslinked rubbery polymer membranes, denoted as PEG/PPG-2CZPIImide (x:y), are prepared from the norbornene-functionalized PEG/PPG oligomer (NB-PEG/PPG-NB) and 2-(bicyclo[2.2.1]hept-5-en-2-ylmethyl)-5,6-di(9H-carbazol-9-yl)isoindoline-1,3-dione (2CZPIImide-NB) via ring-opening metathesis polymerization (ROMP). The molar ratio (x:y) of the NB-PEG/PPG-NB (x) to 2CZPIImide-NB (y) monomers is varied from 10:1 to 6:1. X-ray diffraction (XRD), scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS), and pure gas permeability studies reveal that the comonomer 2CZPIImide-NB successfully increases the *d*-spacing among the crystalline PEG/PPG segments, hence enhancing the diffusivity of gases through the membranes. The synthesized membranes exhibit good CO₂ separation performance, with CO₂ permeabilities ranging from 311.1 to 418.1 Barrer and CO₂/N₂ and CO₂/CH₄ selectivities of 39.4–52.0 and 13.4–16.0, respectively, approaching the 2008 Robeson upper bound. Moreover, PEG/PPG-2CZPIImide (6:1), displaying optimal CO₂ permeability and CO₂/N₂ and CO₂/CH₄ selectivities, shows long-term stability against physical aging and plasticization resistance up to 20 days and 10 atm, respectively.

1. Introduction

Significant attention has been directed toward the conceptualization, advancement, and utilization of membrane processes for gas separation. This can mainly be attributed to the inherent advantages of membranes, such as their relative simplicity, low costs,^[1] good processability,^[2–5] eco-friendliness, and easy scale-up, compared to other gas separation technologies.^[6,7] However, the prevailing limitation of membrane-based gas separation is the well-known “trade-off” between permeability and selectivity for a specific gas,^[8] indicated in the Robeson plot. Nevertheless, this limitation has been overcome via the careful selection of membrane materials; this can be achieved by, for example, fine-tuning the polymer structure, incorporating porous materials, using molecules that can interact with a specific gas, etc.

In the case of polymer membrane-based CO₂ separation, in particular, several advanced materials with specifically designed polymeric structures,^[9] metal–organic frameworks,^[10,11] covalent organic frameworks,^[12] ionic liquids,^[13] and organic–inorganic hybrid structures^[14–16] have been employed to boost CO₂ solubility and/or CO₂ diffusivity across the membrane. Such materials result in membranes with high CO₂ permeability and/or selectivity that surpass Robeson’s upper bound.

Poly(ethylene glycol) (PEG) and its derivatives, possessing ethylene oxide units, are another class of polymers that have been widely used for CO₂ separation due to their high CO₂-philicity.^[17–19] The polar ether groups of PEG exhibit a strong quadruple–quadruple interaction with CO₂ and, hence, have high CO₂ solubility. This then contributes to enhanced selectivity toward CO₂ over other gases. The highly crystalline nature of the ethylene oxide segments, particularly in high-molecular-weight PEG, however, can lead to a reduction in CO₂ permeability.^[20,21]

On the other hand, the low-molecular-weight poly(ethylene glycol)-*block*-poly(propylene glycol) copolymer (PEG/PPG), possessing –CH₃ side chains in the PPG segment, can impede the dense chain packing of the PEG segment, reducing the crystallinity of PEG/PPG and, thereby, expanding its utility in gas

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Fabrication of a high-performance MOF-COP-based porous hollow fiber membrane for carbon dioxide separation

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ABSTRACT

A thin-film nanocomposite (TFNC) hollow fiber membrane with high CO₂ permeability and selectivity was synthesized for the separation of carbon dioxide, a major greenhouse gas. A highly porous polyethersulfone (PES) hollow fiber membrane support with a porous structure was first fabricated using heat and pressure treatments, and a thin film was prepared via interfacial polymerization by reacting polyethylenimine (PEI) with 1,3,5-benzenetricarbonyl trichloride (TMC) on the surface of the PES support. A metal-organic framework (MOF)-covalent organic polymer (COP) complex, obtained via polycondensation between an UiO-66-NH₂ based MOF and a piperazine- and cyanuric chloride-based COP, was then added to the TFNC to prepare the mixed matrix membrane. The complex contributed to reducing defects between the porous support and the coating layer and additionally led to high CO₂ permselectivity by providing a CO₂ permeation pathway within the support. The newly prepared TFNC hollow fiber membrane contributed to its excellent separation performance, as evidenced by a CO₂ permeability of 352 GPU and a CO₂/N₂ ideal selectivity of 75 after optimization of the MOF-COP content.

1. Introduction

CO₂ constitutes the predominant component of greenhouse gases emitted from industrial processes, with approximately 60 % of its emissions stemming from sources associated with energy and power generation. Recent research has suggested the need for net-zero CO₂ emissions to effectively address human-induced global warming, and various efforts have been made toward this goal [1,2]. Notably, power plants, which generate energy from fossil fuels, are often identified as the primary source of CO₂ emissions. Consequently, various studies have investigated effective methods for treating CO₂ from flue gas. Currently, a range of CO₂ treatment methods are under development, including adsorption techniques using solid adsorbents and wet-type absorbents [3]; cryogenic separation, where CO₂ is liquified at low temperatures; and membrane-based separation [4].

Among these approaches, membrane-based separation has proved popular, widely employed for approximately 40 years for not only CO₂ separation but also other applications, including natural gas and

methane refining, and the recovery of hydrogen and oil mist. Its widespread use is attributed to its high energy efficiency, simple operation, and cost-effective and environmental advantages [4].

Among the various types of separation membranes, hollow fiber membranes exhibit exceptional self-supporting performance and flexibility in module fabrication. Moreover, their larger surface area and packing density contribute to enhanced space efficiency and scalability in design [5]. Nevertheless, in order for these membranes to effectively compete with existing gas separation membranes, it is necessary to develop advanced membranes with high permeability and selectivity; membranes composed of thin-film coating layers are currently under investigation in this regard. In general, gas permeability is determined based on the substrate of a membrane and is inversely proportional to the effective thickness of the membrane's selective layer. Simply stated, an increase in the thickness of the selective layer inevitably results in reduced permeability. Moreover, there often exists a distinct trade-off between gas permeability and selectivity in membrane-based gas separation. Thus, extensive research efforts have focused on developing

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Anion Exchange Membranes by Cross-Linking Poly(styrene-*b*-ethylene-*co*-butylene-*b*-styrene) with Poly(aryl piperidinium) Containing Rigid-Flexible Units

Jiyong Choi, Yerim Lee, Haeryang Lim, Hyeonjun Maeng, Taiho Park, and Tae-Hyun Kim*

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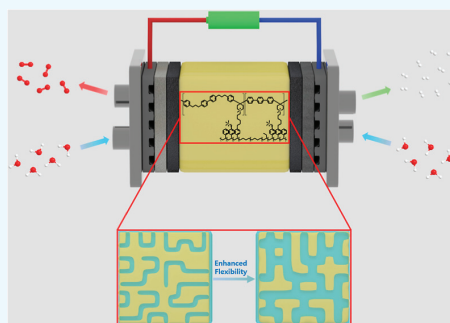
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Supporting Information

ABSTRACT: With the increasing significance of green hydrogen production, anion exchange membrane-based water electrolysis (AEMWE) is gaining prominence. AEMWE with remarkable cell performance and durability has recently been developed, especially that built on poly(aryl piperidinium) (PAP)-based anion exchange membranes due to its high ion conductivity, remarkable chemical stability, and improved mechanical durability. In this work, we developed PAP-based cross-linked poly[(PPBP-*co*-pTP)-SEBS] membranes by chemically cross-linking two polymer components: a copolymer of *para*-terphenyl (pTP) and bis-4,4'-(3-phenylpropyl)biphenyl (PPBP), referred to as PPBP-pTP, and poly(styrene-*b*-ethylene-*co*-butylene-*b*-styrene) (SEBS). Specifically, the structurally rigid pTP was copolymerized with PPBP, containing flexible alkyl chains, forming a unit (PPBP-pTP) similar in structure to SEBS (a polymer combining the rigidity of styrene with the elasticity of ethylene-*b*-butylene). Subsequently, cross-linking PPBP-pTP and SEBS yielded x-(PPBP-pTP)-SEBS membranes with pronounced microphase separation. Additionally, x-(PPBP-15-pTP)-SEBS, with the 15% of PPBP content, exhibited the high OH[−] conductivity (126.71 mS/cm at 80 °C); excellent mechanical properties (tensile strength = 24.91 MPa and elongation at break = 57.59%); exceptional alkaline stability (100% conductivity retention in 2 M KOH at 80 °C after 960 h); and an excellent AEMWE cell performance of 2.67 A/cm² at 1.8 V, using a non-noble catalyst for Ni-based oxygen evolution reaction applications.

KEYWORDS: anion exchange membrane, water electrolysis, polyarylene with rigid-flexible units, non-noble metal catalyst, cross-linking with SEBS



1. INTRODUCTION

In recent years, global energy consumption has steadily risen, driven by population growth and the increasing demands of civilization. An escalating reliance on fossil fuels has increased carbon emissions, further contributing to the degradation of the global environment over time. Against this backdrop, sustainable renewable energy sources are urgently needed.^{1–3} Hydrogen energy, primarily implemented by fuel cells and water electrolysis (WE), is one of the most promising next-generation energy solutions to replace fossil fuels. It is eco-friendly and sustainable, with high energy density and no carbon emissions.^{4–6} These advantages have fueled an increasing demand for green hydrogen production using WE.

Various WE methods have been proposed: alkaline water electrolysis uses an alkaline solution as an electrolyte to electrolyze water into hydrogen and oxygen. This method has an economic advantage because it can use inexpensive, non-noble metal catalysts. However, the hydrogen production rate is relatively slow, and the produced gases crossover through the porous membrane separating the two electrodes, which has the disadvantage of lowering the purity of the hydrogen produced.^{7,8}

Another WE method, proton exchange membrane-based water electrolysis (PEMWE), which uses a proton exchange membrane (PEM) both as the electrolyte and the separator, can suppress gas crossover and shows high cell performance because it conducts H⁺, which has higher mobility than OH[−].^{9,10} However, it is expensive because PEMWE uses a perfluorinated polymer as the PEM, along with a precious metal catalyst.

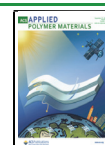
A third method, anion exchange membrane-based water electrolysis (AEMWE), has recently received a great deal of research attention because AEMWE operates in alkaline conditions, allowing the use of low-cost catalysts, such as Ni, Co, and Mo. Moreover, using polymer electrolyte membranes based on nonfluorinated hydrocarbons significantly increases

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A Polysaccharide Binder with Carbon Quantum Dots for Improved Flexibility of Si Anodes in Lithium–Ion Batteries

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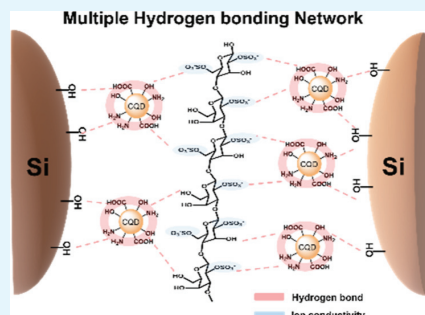
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Supporting Information

ABSTRACT: A polymer binder with high adhesive strength and flexible mechanical properties was developed by introducing carbon quantum dots (CQDs) into carrageenan (CGN), a type of polysaccharide obtained from marine red algae. The 350 nm silicon-based electrode containing CGN-CQD as a binder showed improved cell performance compared to Si@CGN, fabricated with CGN binder, due to the improved adhesive property and enhanced flexibility of CGN-CQD. These properties were due to the formation of multiple hydrogen-bonding networks between the numerous polar functional groups of the CQDs and the CGN. This 3D network imparted flexible properties to the electrode, accommodating the irreversible volume changes of Si that occur during charging and discharging and demonstrating high electrode integrity. The optimized electrode, Si@CGN-CQDs, has 5 wt % CQD relative to CGN and exhibited an initial discharge capacity of 3486 mA h g⁻¹ at a current density of 0.2 C and a high capacity retention of 61.7% after 100 cycles. This is a significantly superior performance to the Si@CGN electrode, which showed an initial discharge capacity of 3205.5 mA h g⁻¹ and capacity retention of only 12.2% after 100 cycles. Furthermore, the difference in capacity retention becomes more evident at a higher current density of 0.5 C. That is, the Si@CGN electrode showed a capacity retention of 4% after 100 cycles, while the Si@CGN-CQDs electrode showed a capacity retention of 48%. The CGN-CQD binder exhibits electrode integrity even at higher charge and discharge rate of 0.5 C due to its improved flexibility and mechanical properties.

KEYWORDS: carbon quantum dot, carrageenan, adhesion, 3D network, elasticity, silicon anode binder, binder additive



INTRODUCTION

With their extensive use in electric vehicles and energy storage systems, high-energy density lithium–ion batteries (LIBs) are increasingly central to society. Although many attempts have been made to develop LIBs with improved energy density, significant breakthroughs have yet to occur due to the low theoretical capacity (273 mA h g⁻¹) of graphite, widely used as an anode active material. Silicon (Si) is a promising alternative.

Due to its high theoretical capacity (4200 mA h g⁻¹), low operating voltage window (0.4 V), and abundance, Si is one of the most promising next-generation anode active materials. However, Si typically undergoes significant volume expansion of up to 400% during the charge and discharge cycle. The Si particles may be pulverized, and consequently, the physical contact network between the active material and the conducting agent collapses, resulting in rapid capacity degradation. These limitations are barriers to the commercialization of Si anodes. In response, this problem has been addressed in various ways, including the use of nanosized (<150 nm) Si particles, the critical threshold beyond which the volume expansion of Si is maximized.¹ Additionally, the nature of Si has been modified by incorporating metallic particles^{2–5} or through carbon doping.^{6–9} However, issues associated with

process costs and volume expansion have not been sufficiently addressed.

The binder constitutes only a fraction of the electrode. However, it plays a critical role in connecting the active material and the conducting agent, facilitating the delivery of Li ions and electrons generated during electrochemical reactions while also ensuring adhesion between the electrode and the current collector, contributing significantly to the structural integrity of the electrodes. It also plays a significant role in protecting electrode structures against volume expansion in Si anodes, preventing the rapid degradation of electrode performance; numerous studies have developed novel binders for Si active materials.^{10–13}

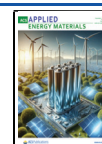
For example, poly(vinylidene fluoride), or PVdF, is widely used as a cathode material but is considered unsuitable as a Si anode binder due to its poor mechanical properties. PVdF also

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Oxidized λ -Carrageenan as an Aqueous Silicon-Anode Polymer Binder with Reduced Viscosity for Lithium-Ion Batteries

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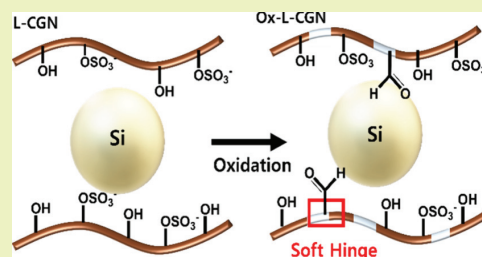
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Supporting Information

ABSTRACT: λ -Carrageenan (L-CGN) exhibits high potential as a Si-anode binder due to its high adhesive strength and 3D structure with Si. This study aimed to improve the mechanical properties and reduce the high viscosity of polysaccharides, including L-CGN, by oxidation. The aldehyde functional group introduced via oxidation served as a hinge, providing flexibility to relatively stiff polysaccharide binders and strengthening their interactions with the Si active materials. The oxidized CGN binders exhibited enhanced adhesion and mechanical strength, thereby maintaining the electrode integrity against Si's volume expansion. The Si@Ox-L-CGN electrode, combining oxidized L-CGN (Ox-L-CGN) as a binder and 350 nm-sized Si as an active material, displayed enhanced mechanical properties compared to those of the Si@L-CGN electrode. Si@Ox-L-CGN-S, oxidized by 5% relative to Si@L-CGN, showed an improved initial Coulombic efficiency of 78.6% compared to that of Si@L-CGN (72.6%). Furthermore, at a Si loading level of 1.0 mg cm⁻², Si@Ox-L-CGN-S displayed a capacity of 2032 mAh g⁻¹ after 100 cycles (compared to a very low value of 248 mAh g⁻¹ for Si@L-CGN) and a high capacity retention of 73.9%. The results highlight the potential of Ox-L-CGN as a binder for Si anodes with a large particle size and a high loading level.

KEYWORDS: Oxidation of λ -carrageenan, Polymer binder, Silicon anode, Lithium-ion batteries, Reduced viscosity



INTRODUCTION

Advances in renewable energy technologies are critical for the sustainable development of our society. However, the intermittent nature of renewable energy sources requires complementary energy storage technologies, such as rechargeable batteries, to ensure continuous and reliable power supplies. Among electrochemical energy storage devices, lithium-ion batteries (LIBs) have extensive applications in portable electronic devices, electric vehicles, and grid-scale energy storage systems due to their high energy density, power density, and stable cycle life characteristics. However, given the increasing demands of electric vehicles and various other applications, it is essential to develop next-generation LIBs with a higher energy density and longer cycle life.

The electrodes, particularly the anode material, greatly influence the performance of the LIBs. During the charging and discharging cycling processes, the anode stores and releases Li ions. To obtain a LIB with a high energy density, an anode material must have a high theoretical capacity and be unreactive in the electrolyte to maintain a high Coulombic efficiency and battery capacity.

Silicon is a potential anode material for next-generation LIBs due to its outstanding theoretical specific capacity (4200 mAh g⁻¹, Li_{4.4}Si), low operating voltage (<0.5 V vs Li⁺/Li), and natural abundance. However, the significant volume expansion

of silicon associated with lithiation (>400% for Li_{4.4}Si) and the repeated volume changes with cycling, lead to pulverization of silicon particles, loss of electrical contact between electrode materials, and the formation of an unstable solid electrolyte interphase (SEI) layer. This causes rapid degradation of electrode performance, which has severely impeded the wider application of silicon anodes in high energy density LIBs.¹

The negative impact of the pulverization of silicon nanoparticles during the charge/discharge process becomes particularly severe when the Si particle size is over 150 nm or the percolation size.^{2,3} For this reason, most studies on Si anodes use Si nanoparticles mostly smaller than 100 nm, despite the fact that larger nanoparticles can improve the energy density and reduce cost, making them more suitable for industrial-scale applications.

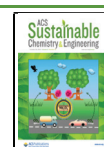
To address the issues related to silicon as an anode, material modifications have been explored, such as nanostructured silicon,^{4,5} porous structured silicon,^{6,7} or Si/C composites.^{8–11}

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Metal-Coordinated, Dual-Crosslinked PIM Polymer Membranes for Upgraded CO₂ Separation: Aging and Plasticization Resistance

Iqbal Hossain, Kwan Il Kim, Asmaul Husna, Jun Hyeok Kang, Tae-Hyun Kim,* and Ho Bum Park*

The practical use of polymers of intrinsic microporosity (PIMs) in CO₂ separation is often hindered by their moderate selectivity, performance instability over time, and pressure constraints. To address these limitations, a straightforward approach is presented to enhance the CO₂ separation capability of PIM-1 by incorporating metal ions into uniformly hydrolyzed PIM-1 (cPIM). This dual linking strategy, achieved via ionic and coordination bonding of metal ions with the polymeric side chains including —COOH and —CONH₂, restructures the polymer, disrupting hydrogen bonds between cPIM chains and creating active sites for CO₂ via π -complexation. This modification enhances gas permeability while maintaining high selectivity. The optimized zinc-coordinated membrane achieves an impressive CO₂ permeability of $\approx 2,500$ Barrer with CO₂/N₂ and CO₂/CH₄ selectivities of 27.1 and 23, respectively, outperforming pristine cPIM (700 Barrer; CO₂/N₂ = 27; CO₂/CH₄ = 19). Notably, this performance surpasses the 2008 Robeson upper-bound limits for both gas pairs. Additionally, the metal-coordinated membranes exhibit remarkable long-term stability, resisting aging effects for up to 20 days and maintaining anti-plasticization properties at pressures up to 20 bar. These dual-crosslinked membranes demonstrate promising potential for mixed gas separation, indicating their suitability for real-world industrial applications.

1. Introduction

Capturing CO₂ from flue gas or natural gas is vital for maintaining atmospheric CO₂ levels and meeting regulatory requirements for a sustainable future.^[1,2] Among various CO₂ separation technologies, membrane-based systems have garnered significant attention due to their advantages over conventional methods like cryogenic separation, pressure swing adsorption, and chemical scrubbing, offering lower energy consumption, scalability, operational simplicity, and a compact footprint.^[3–6] However, key challenges such as membrane stability and the inherent trade-off between permeability and selectivity in traditional polymers limit their broader adoption.^[7,8]

Polymers of intrinsic microporosity (PIM-1) represent a significant leap forward in membrane technology for gas separation. PIM-1 membranes are particularly promising for CO₂ capture from CH₄ and N₂ due to their high permeability, ranging from 2,300 to 18,000 Barrer, surpassing the 1991 Robeson upper bound for the permeability-selectivity trade-off.^[9–13] This ultrahigh permeability is attributed to the large free volume and interconnected

porosity of PIM-1, which also boasts a high surface area (≈ 850 m² g^{−1}).^[9,10] The rigid and contorted molecular structure of PIM-1 restricts chain mobility, resulting in a frustrated packing arrangement that yields intrinsic microporosity.^[13] Despite these benefits, PIM-1 membranes suffer from moderate CO₂ selectivity over N₂ and CH₄ and experience rapid aging as the polymer chains thermally relax into more compact configurations, reducing free volume and permeability over time.^[7,14,15] Additionally, their performance is sensitive to applied pressure, with swelling and pressure-induced densification reducing effectiveness.^[11] Overcoming these issues is essential for realizing the full potential of PIM-1 membranes in real-world applications.

Various chemical modifications of PIM-1's nitrile groups to create amides,^[16,17] amines,^[18] tetrazoles,^[19] and other functional groups^[20–28] have been explored to enhance gas separation performance. Carboxylic acid-functionalized PIM-1 (cPIM) is particularly noteworthy for its improved CO₂ sorption and diffusion

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Si anodes via dual strategies of coating Si with a rigid polymer and employing a polymer binder with improved mechanical properties

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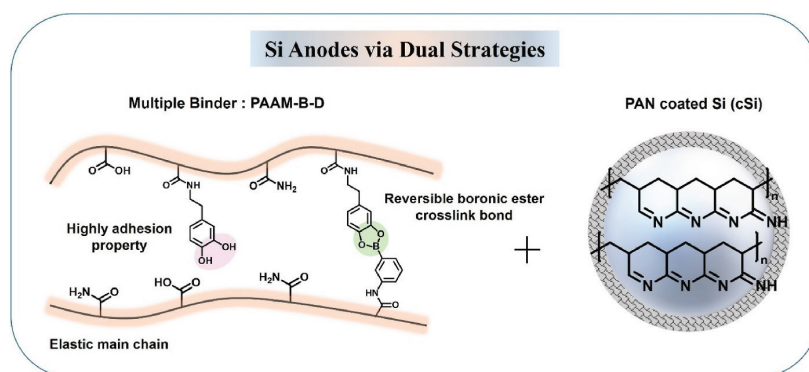
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HIGHLIGHTS

- Si anode operation beyond critical size (350 nm Si) through dual strategy.
- Coating the Si particles with a rigid polymer helped suppress volume change.
- A polymer binder with improved elasticity relieved stress during volume expansion.
- The cSi@PAAM-B-D shows high cycle performance and rate capability.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

350-Nm Si
Hardness
Elastic property
Water soluble binder
Coating si

ABSTRACT

Si undergoes significant volume change over cycles which degrades the structural integrity and stability of the electrode. This volume change is the primary barrier to the commercialization of Si anodes, and it is more pronounced for Si particle sizes over 150 nm. In this study, a crosslinked polymer binder is developed using poly (acrylic acid-co-acrylamide) (PAAM) with enhanced elasticity compared to the widely used poly(acrylic acid). PAAM is further grafted with boronic acid and dopamine, yielding PAAM-B-D, a binder with improved adhesion due to its 3D crosslinked network. Additionally, 350-nm Si is coated with cyclized polyacrylonitrile (cPAN) and heat-treated to form a conjugated structure. The cPAN-coated Si (cSi) exhibits enhanced conductivity and mechanical stiffness and is used as an active material. The developed Si anode effectively combines cPAN-coated Si with the crosslinked network formed in the PAAM-B-D polymer for enhanced adhesion. The cSi@PAAM-B-D electrode sufficiently maintains its structural integrity and mitigates the Si volume change even with the large-sized 350-nm Si. The cSi@PAAM-B-D exhibits a high initial Coulombic efficiency of 86.5 %, at a Si mass

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Thermal Debromination-Induced Cross-Linking of PIM-Polyimide Membranes: Improved CO₂ Gas Permeability, Selectivity, and Separation Performance

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ARTICLE INFO

Editor: Gaohong He

Keywords:

Gas separation
Bromination–debromination
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Anti-plasticization
Anti-aging

ABSTRACT

Demand for advanced polymers with intrinsic microporosity (PIM) is growing due to their enhanced free volume and potential in gas separation membranes. However, PIM-based membranes are vulnerable to aging effects and loss of stable performance. To overcome this drawback, we synthesized a PIM-polyimide (PI) polymer based on PIM-dianhydride and a durenne diamine unit (PIM-PI-1). Bromination at the PIM-PI-1 benzyl positions provided PIM-PI-Br-Z (bromination degree Z = 15, 25, and 50 %), precursors for thermal debromination-induced cross-linking. The optimal cross-linked membrane derived from 50 % brominated PIM-PI-Br-50 demonstrated remarkable CO₂ permeability (5401 Barrer) combined with good CO₂/CH₄ (21.1) and CO₂/N₂ (21.0) selectivity, surpassing the 2008 upper bound. Additionally, the membrane demonstrated good performance in mixed gas tests (CO₂/N₂), exhibiting a CO₂ permeability of 2299 Barrer and CO₂/N₂ selectivity of 31. The thermally cross-linked membrane exhibited promising anti-aging properties up to 13 days and excellent CO₂-induced plasticization resistance even under pressures up to 18 atm, demonstrating its potential for high-performance PIM-based gas separation membranes.

1. Introduction

Advances in carbon capture, storage, and utilization (CCSU) technology are essential for mitigating carbon dioxide (CO₂) emissions and slowing the pace of global warming [1–4]. Numerous techniques for CO₂ capture technology have been devised, including cryogenic distillation, adsorption, chemical absorption, and membrane separation [5–8]. The current pursuit of novel, energy-efficient CO₂ capture is driving the development of membrane-based separation processes with low energy consumption, scalability, cost-effectiveness, small carbon footprint, ease of operation, and compact module formation [9–11].

Within the spectrum of potential gas separation techniques, various membrane materials have been developed, including inorganic, polymeric, and polymer-inorganic mixed matrix membranes (MMMs) [12–18]. Among these, polymer membranes have attracted particular attention due to their excellent film-forming ability, good thermal and

mechanical properties, economical production, and straightforward manufacturing processes [19–21]. Numerous studies have explored the development of new polymer membrane materials for CO₂ separation. However, materials that can satisfy both processability and price constraints, as well as gas separation performance, are still limited to polyimides (PIs), polysulfones, and cellulose acetates. Among these, PIs have been the most extensively studied due to their excellent chemical, mechanical, and thermal properties, the advantages mentioned above, and the relative ease of preparing a range of chemical structures [22–25].

Nevertheless, challenges remain before these PI-based polymer membranes can be used commercially. The Robeson upper bound is a technical limit between permeability and selectivity, meaning a highly permeable membrane will have a low selectivity, and vice versa. Most polymer membranes for CO₂ separation, including PIs, fail to overcome this trade-off. As a result, several approaches have been explored to

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ARTICLE

Comparing kinetic methods for ball-mill grinding: Molecular weight versus refractive index-based methods

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Funding information

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Abstract

Polymers are commonly subjected to ball-mill grinding (BMG) to induce chain scission. Milling and polymer parameters can influence the kinetics of degradation, and various kinetic methods have been employed to study this process. The most common methods include molecular weight models (fitting the decrease in molecular weight with milling time) and refractive index (RI)-based methods (such as the Florea method, which fits the decrease in RI signal with milling time). In this report, we compare the rate constant trends obtained from three different molecular weight models and the Florea method to provide a deeper understanding of how the kinetic method employed in kinetic studies influences the observed reactivity trends. Specifically, each kinetic method was applied to BMG-induced degradation data from polystyrene (PS) and poly(lactide) (PLA) of varying initial molecular weight. Each kinetic method produced different rate constant trends (e.g., initial molecular weight vs. rate constant), which could also be influenced by changing the milling duration. Molecular weight models or the Florea method were better suited with longer or shorter milling durations, respectively. Furthermore, the rate constant trends for PS and PLA were relatively consistent if compared with the same kinetic method. We expect that this work will help guide future BMG-based degradation kinetics studies.

KEYWORDS

ball-mill grinding, chain scission, kinetics, mechanochemistry, polymer degradation

INTRODUCTION

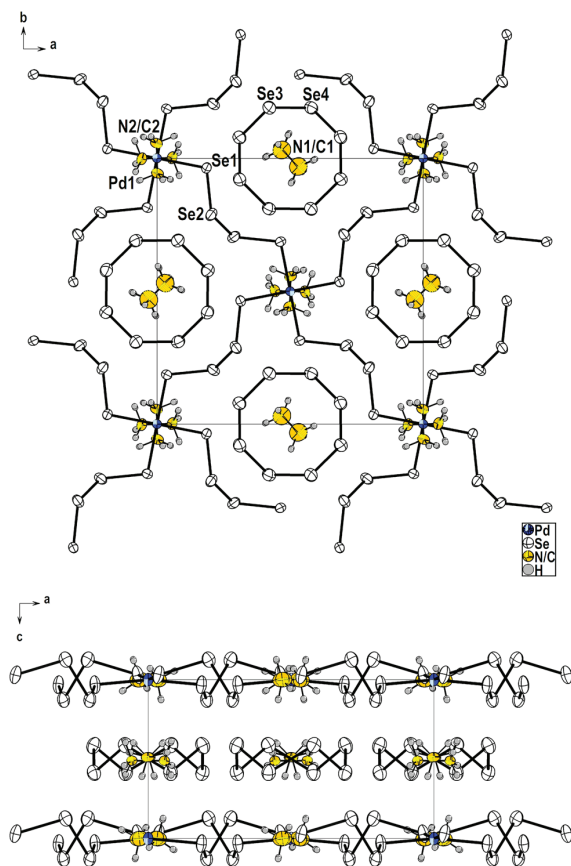
When polymers are subjected to ball-mill grinding (BMG), various transformations can occur,¹ with one of the most important being chain scission. This phenomenon can be exploited for various applications including polymer degradation,^{2,3} depolymerization,^{4,5} mechano-phore activation,^{6,7} chain-end functionalization,^{8,9} and block copolymer synthesis.^{10,11} Therefore, increasing effort has been devoted to understanding how ball milling^{12–14} and polymer parameters^{15–17} influence chain scission. A better understanding of these parameters can also be useful in limiting chain scission in applications in which degradation is an unwanted side reaction, such as in the mechanochemical synthesis^{18–20} and post-polymerization modification of polymers.^{21,22} A common method of evaluating the influence of

parameters is to compare degradation rates from experiments that only differ by the parameter of interest. Polymer degradation is often monitored by size-exclusion chromatography (SEC) and changes in the molecular weight or refractive index (RI) can be used to calculate degradation rate constants.

Chain scission occurs for polymers with a molecular weight that exceeds a limiting molecular weight (M_{lim}). The parent polymer is cleaved into daughter fragments and the number-average molecular weight (M_n) of the sample decreases with increasing milling time. Molecular weight models are phenomenological models that can be used to predict the molecular weight after a given degradation time or can be used to fit degradation data and extract a degradation rate constant (k).^{23–29} While these models were developed for other degradation processes, such as ultrasonication, we recently evaluated and

Kang-Woo Kim* and Jong-Min Noh

Crystal structure of bis(methylammonium) hexadecaselenidopalladate(II), $(\text{CH}_3\text{NH}_3)_2\text{PdSe}_{16}$

**Table 1:** Data collection and handling.

Crystal:	Black chunk
Size:	0.18 × 0.15 × 0.12 mm
Wavelength:	Mo K α radiation (0.71073 Å)
μ :	25.9 mm ⁻¹
Diffractometer, scan mode:	PHOTON 100 CMOS, φ and ω
θ_{max} , completeness:	28.3°, >99 %
$N(hkl)_{\text{measured}}$, $N(hkl)_{\text{unique}}$, R_{int} :	40,693, 1457, 0.099
Criterion for I_{obs} , $N(hkl)_{\text{gt}}$:	$I_{\text{obs}} > 2 \sigma(I_{\text{obs}})$, 1377
$N(\text{param})_{\text{refined}}$:	54
Programs:	Bruker [1], SHELX [2], WinGX/ORTEP [3], Diamond [4]

CCDC no.: 2341033

Table 1 contains crystallographic data and Table 2 contains the list of the atoms including atomic coordinates and displacement parameters.

1 Source of material

K_2PdCl_4 (0.020 g, 0.061 mmol), K_2Se_4 (0.096 g, 0.24 mmol) and $\text{CH}_3\text{NH}_3\text{Cl}$ (0.008 g, 0.12 mmol) were charged to a Pyrex tube with diameter of 9 mm under an argon atmosphere and about 0.5 mL methanol was added as a solvent. While the solvent was being frozen, the Pyrex tube was evacuated under vacuum and sealed with the use of a flame. The sealed tube was placed in an oven and heated at 80 °C for 3 days, then cooled to room temperature. Black chunky crystals were isolated by filtration and washed with methanol and diethyl ether several times. Crystals of $(\text{CH}_3\text{NH}_3)_2\text{PdSe}_{16}$ were obtained in 11 % yield, based on the Pd metal used.

2 Experimental details

H atoms were positioned geometrically and treated as riding, with N–H = 0.90 Å and C–H = 0.97 Å; $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{N})$ and $1.5U_{\text{eq}}(\text{C})$. H atoms of NH_3 and CH_3 were positioned to be staggered with respect to the shortest other bond to the atom to which NH_3 and CH_3 is attached.

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Abstract

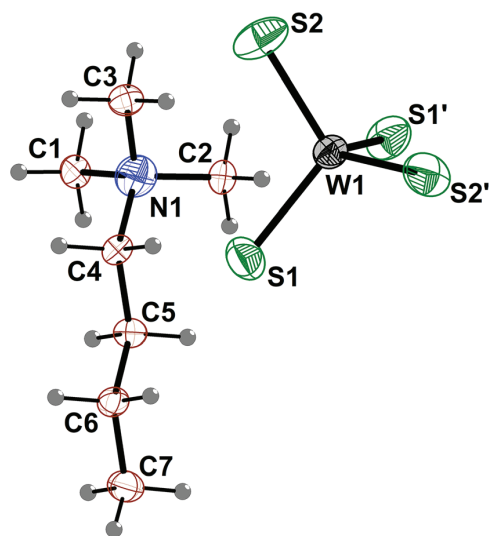
$(\text{CH}_3\text{NH}_3)_2\text{PdSe}_{16}$, tetragonal, $P\bar{4}b2$ (no. 117), $a = 12.7821(4)$ Å, $c = 7.1123(2)$ Å, $V = 1162.02(8)$ Å³, $Z = 2$, $R_{\text{gt}}(F) = 0.0306$, $wR_{\text{ref}}(F^2) = 0.0841$, $T = 223$ K.

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Kang-Woo Kim* and Young-Hyun Yoo

Crystal structure of bis(*N,N,N*-trimethylbutanaminium) tetrathiotungstate(VI), (BuMe₃N)₂[WS₄]

**Table 1:** Data collection and handling.

Crystal:	Yellow block
Size:	0.32 × 0.22 × 0.21 mm
Wavelength:	Mo K α radiation (0.71073 Å)
μ :	5.60 mm ⁻¹
Diffractometer, scan mode:	PHOTON III M14, φ and ω
$\theta_{\max}$, completeness:	28.3°, 98 %
$N(hkl)_{\text{measured}}$, $N(hkl)_{\text{unique}}$:	14,425, 2701, 0.046
R_{int} :	
Criterion for I_{obs} , $N(hkl)_{\text{gt}}$:	$I_{\text{obs}} > 2\sigma(I_{\text{obs}})$, 2659
$N(\text{param})_{\text{refined}}$:	97
Programs:	Bruker, ¹ SHELX, ² WinGX/ORTEP, ³ Diamond ⁴

1 Source of material

(NH₄)₂WS₄ (0.030 g, 0.086 mmol) and BuMe₃NI (0.063 g, 0.26 mmol) were charged to a Pyrex tube with diameter of 9 mm and about 0.3 mL 1:2 (v/v) H₂O/MeOH mixture was added as a solvent. While the solvent was being frozen, the Pyrex tube was evacuated under vacuum and sealed with the use of a flame. The sealed tube was placed in an oven and heated at 90 °C for a day, then cooled to room temperature. Yellow orange polyhedral crystals were isolated by filtration and washed with MeOH and diethyl ether several times. Crystals of (BuMe₃N)₂[WS₄] were obtained in 13 % yield, based on the W metal used.

2 Experimental details

H atoms were positioned geometrically and treated as riding, with C–H = 0.98 (CH₂) and 0.97 (CH₃) Å with $U_{\text{iso}}(\text{H}) = 1.2$ (1.5 for methyl) $U_{\text{eq}}(\text{C})$. H atoms of the CH₃ were positioned to be staggered with respect to the shortest other bond to the atom to which the CH₃ is attached.

3 Comment

The title compound, (BuMe₃N)₂[WS₄], prepared by the solvothermal reaction of (NH₄)₂WS₄ and BuMe₃NI with a 1:2 (v/v)

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published online April 22, 2024

Abstract

C₁₄H₃₆N₂S₄W, monoclinic, *C*2/*c* (no. 15), $a = 16.175(3)$ Å, $b = 9.7755(15)$ Å, $c = 15.452(3)$ Å, $\beta = 115.183(7)^\circ$, $V = 2211.0(7)$ Å³, $Z = 4$, $R_{\text{gt}}(F) = 0.0190$, $wR_{\text{ref}}(F^2) = 0.0490$, $T = 223$ K.

CCDC no.: 2347476

The molecular structure is shown in the figure. Table 1 contains crystallographic data and Table 2 contains the list of the atoms including atomic coordinates and displacement parameters.

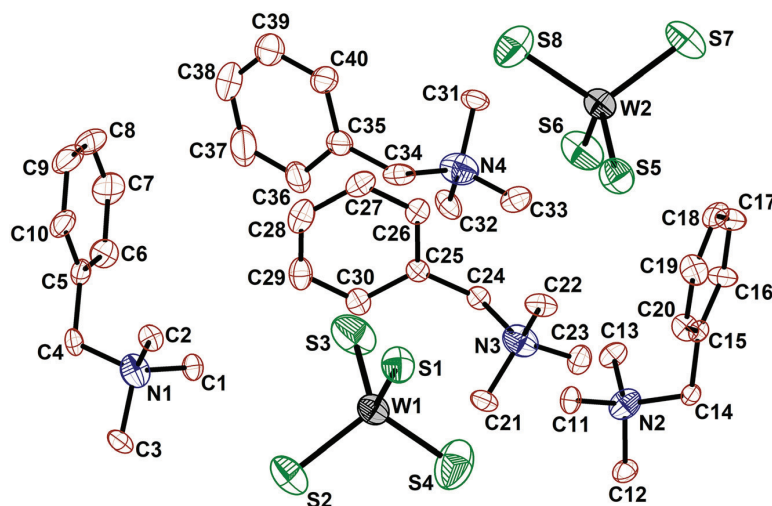
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Kang-Woo Kim* and Sunghyeon Kim

Crystal structure of bis(benzyltrimethylammonium) tetrathiotungstate(VI), $\{(\text{C}_6\text{H}_5\text{CH}_2)(\text{CH}_3)_3\text{N}\}_2[\text{WS}_4]$



<https://doi.org/10.1515/ncrs-2024-0228>

Received May 27, 2024; accepted July 2, 2024;
published online July 23, 2024

Abstract

$\text{C}_{20}\text{H}_{32}\text{N}_2\text{S}_4\text{W}$, monoclinic, $C2/c$ (no. 15), $a = 37.4033(15) \text{ \AA}$, $b = 17.2676(7) \text{ \AA}$, $c = 16.4860(6) \text{ \AA}$, $\beta = 109.319(1)^\circ$, $V = 10,048.2(7) \text{ \AA}^3$, $Z = 16$, $R_{\text{gt}}(F) = 0.0422$, $wR_{\text{ref}}(F^2) = 0.0930$, $T = 223 \text{ K}$.

CCDC no.: 2367191

Table 1 contains crystallographic data and Table 2 contains the list of the atoms including atomic coordinates and displacement parameters.

Table 1: Data collection and handling.

Crystal:	Yellow block
Size:	$0.29 \times 0.14 \times 0.08 \text{ mm}$
Wavelength:	Mo $K\alpha$ radiation ($0.71073 \text{ \AA}$)
μ :	4.94 mm^{-1}
Diffractometer, scan mode:	Bruker APEX-II, φ and ω
θ_{max} , completeness:	28.3° , $>99\%$
$N(hkl)_{\text{measured}}$, $N(hkl)_{\text{unique}}$, R_{int} :	118,320, 12,469, 0.079
Criterion for I_{obs} , $N(hkl)_{\text{gt}}$:	$I_{\text{obs}} > 2\sigma(I_{\text{obs}})$, 8,148
$N(\text{param})_{\text{refined}}$:	487
Programs:	Bruker, ¹ SHELX ² WinGX/ORTEP, ³ Diamond ⁴

1 Source of material

$(\text{NH}_4)_2\text{WS}_4$ (0.010 g, 0.029 mmol) and $\text{BenzylMe}_3\text{NCl}$ (0.016 g, 0.086 mmol) were charged to a Pyrex tube with diameter of 9 mm and about 0.5 mL 1:4 (v/v) $\text{H}_2\text{O}/\text{MeOH}$ mixture was added as a solvent. While the solvent was being frozen, the Pyrex tube was evacuated under vacuum and sealed with the use of a flame. The sealed tube was placed in an oven and heated at 80°C for a day, then cooled to room temperature. Yellow orange crystals were isolated by filtration and

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Article

***Triglochin maritima* Extracts Exert Anti-Melanogenic Properties via the CREB/MAPK Pathway in B16F10 Cells**

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Abstract: *Triglochin maritima*, a salt-tolerant plant, has demonstrated antioxidant effects, the ability to prevent prostate enlargement, antifungal properties, and skin moisturizing benefits. This study aimed to explore the anti-melanogenic potential of the 70% ethanol extract of *T. maritima* (TME) along with its ethyl acetate (TME-EA) and water (TME-A) fractions. TME (10–200 µg/mL), TME-EA (1–15 µg/mL), and TME-A (100–1000 µg/mL) were prepared and applied to B16F10 cells with or without α-MSH for 72 h. MTT assays were used to assess cytotoxicity, and anti-melanogenesis activity was determined by measuring melanin content, conducting a tyrosinase activity assay, and evaluating the expression of melanogenesis-related genes and proteins via RT-PCR and Western blotting. HPLC-PDA was used to analyze TME and TME-EA. The IC₂₀ cytotoxicity values of TME, TME-A, and TME-EA without α-MSH, were 198.426 µg/mL, 1000 µg/mL, and 18.403 µg/mL, respectively. TME and TME-EA significantly decreased melanin and tyrosinase activity in α-MSH-stimulated B16F10 cells, with TME-EA showing comparable effects to arbutin, while TME-A showed no influence. TME-EA down-regulated melanogenesis genes (*Tyr*, *Trp1*, *Dct*, *Mitf*, *Mc1r*) and reduced CREB, p-38, and JNK phosphorylation while increasing ERK phosphorylation, suggesting the CREB/MAPK pathway's role in the anti-melanogenic effect. Luteolin was identified as a potential active ingredient. TME-EA may serve as an effective cosmeceutical for hyperpigmentation improvement due to its anti-melanogenic properties.

Keywords: *Triglochin maritima*; halophyte; anti-melanogenesis; tyrosinase; CREB/MAPK pathway; luteolin



Citation: Lee, W.-H.; Ha, Y.; Park, J.-I.; Joh, W.B.; Park, M.; Kim, J.K.; Jeon, H.-K.; Kim, Y.-J. *Triglochin maritima* Extracts Exert Anti-Melanogenic Properties via the CREB/MAPK Pathway in B16F10 Cells. *Mar. Drugs* **2024**, *22*, 532. <https://doi.org/10.3390/md22120532>

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1. Introduction

Melanocytes are skin cells that synthesize melanin pigments in melanosomes and transport melanin to neighboring keratinocytes through their dendrites [1]. While the quantity of melanin pigment determines skin shade, melanocytes also protect human skin from ultraviolet (UV) rays and various environmental stresses [2,3]. However, excessive production of melanin can cause skin pigmentation diseases, including melasma, age spots, freckles, and malignant melanoma [4]. Meanwhile, the incidence of hyperpigmentation has increased owing to environmental pollution associated with rapid industrialization and excessive exposure to UV and pollutants, leading to alterations in cytokine and hormone production [5,6].

Melanin synthesis occurs in melanosomes. More specifically, eumelanin (brownish black) and pheomelanin (reddish yellow) are produced in melanocytes, a process that is mediated by tyrosinase, tyrosinase-related protein 1 (TRP-1), and dopachrome tautomerase (DCT) [7]. The initial step in the synthesis of eumelanin and pheomelanin involves the

Article

De Novo Genome Assembly of the Sea Star *Patiria pectinifera* (Muller & Troschel, 1842) Using Oxford Nanopore Technology and Illumina Platforms

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Abstract: The sea star *Patiria pectinifera* (Asteroidea; Asterinidae; homotypic synonym: *Asterina pectinifera*; Muller & Troschel, 1842) is widely distributed in the coastal regions of the Seas of East Asia and the northern Pacific Ocean. Here, a de novo genome sequence of *P. pectinifera* as a reference for fundamental and applied research was constructed by employing a combination of long-read Oxford Nanopore Technology (ONT) PromethION, short-read Illumina platforms, and 10 × Genomics. The draft genome of *P. pectinifera*, containing 13,848,344 and 156,878,348 contigs from ONT and Illumina platforms, respectively, was obtained. Assembly with CANU resulted in 2262 contigs with an N50 length of 367 kb. Finally, ARCS + LINKS assembly combined these contigs into 328 scaffolds, totaling 499 Mb with an N50 length of 2 Mbp. The estimated genome size by GenomeScope analysis was 461 Mb. BUSCO analysis indicated that 930 (97.5%) of the expected genes were found in the assembly, with 889 (93.2%) being single-copy and 41 (4.3%) duplicated after searching against the metazoan database. Annotation, utilizing sequences obtained from Illumina RNA-Seq and Pacific Biosciences Iso-Seq, led to the identification of 22,367 protein-coding genes. When examining the orthologous relationship of *P. pectinifera* against the scaffolds of the common sea star *Patiria miniata*, high contiguity was observed. Annotation of repeat elements highlighted an enrichment of 1,121,079 transposable elements, constituting 47% of the genome, suggesting their potential role in shaping the genome structure of *P. pectinifera*. This de novo genome assembly is expected to be a valuable resource for future studies, providing insight into the developmental, environmental, and ecological aspects of *P. pectinifera* biology.

Keywords: Asteroidea; sea star genome; starfish; *Patiria pectinifera*; de novo genome assembly



Citation: Rhee, J.-S.; Nam, S.-E.; Lee, S.J.; Park, H. De Novo Genome Assembly of the Sea Star *Patiria pectinifera* (Muller & Troschel, 1842) Using Oxford Nanopore Technology and Illumina Platforms. *Diversity* **2024**, *16*, 91. <https://doi.org/10.3390/d16020091>

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1. Introduction

The Asteroidea, commonly known as sea stars or starfishes, constitutes one of the largest and most distinctive classes in the phylum Echinodermata, which has four additional other well-defined clades: Crinoidea (sea lilies and feather stars), Echinoidea (sea urchins, sand dollars, and sea biscuits), Holothuroidea (sea cucumbers), and Ophiuroidea (basket stars and brittle stars) [1,2]. Sea stars are widely distributed throughout the oceans, occupying various depths, and play a crucial role as highly predatory scavengers of benthic macrofauna, engaging in grazing activities. They exhibit remarkable diversity in terms of phenotypes, displaying extraordinary coloration, varying sizes, and responses to environmental conditions. The life cycle of sea stars involves a unique metamorphosis during growth, transitioning from a bilateral symmetric planktonic larval stage to settling as radial symmetric adults. Noteworthy for their longevity, ubiquity, prolificacy, prevalence, dominance, possession of calcified skin for protection, and phenotypic plasticity, changes in the



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Research article

Acute and chronic effects of triclosan on the behavior, physiology, and multigenerational characteristics of the water flea *Moina macrocopa*Seong Duk Do^{a,1}, Md. Niamul Haque^{a,b,1}, Jaehee Kim^a, Dong-Hoon Im^c, Jae-Sung Rhee^{a,b,d,*}^a Department of Marine Science, College of Natural Sciences, Incheon National University, Incheon 22012, South Korea^b Research Institute of Basic Sciences, Incheon National University, Incheon 22012, South Korea^c Marine Environment Research Division, National Institute of Fisheries Science, Busan 46083, South Korea^d Yellow Sea Research Institute, Incheon 22012, South Korea

ARTICLE INFO

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Triclosan
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Neurotoxicity
Oxidative stress

ABSTRACT

Triclosan, a chlorinated biphenyl ether is widely used in industrial products and cosmetics due to its antibiotic activity. Although relatively levels of triclosan have been detected in aquatic ecosystems, limited information is available regarding the acute and chronic impacts of triclosan on aquatic invertebrates, especially planktonic crustaceans. In this study, we analyzed the acute (24 h) and chronic (14 days exposure across three generations) effects of different concentrations of triclosan [1/10 of the no observed effect concentration (NOEC), the NOEC, and 1/10 of the LC50] calculated from the 24 h acute toxicity value, on the water flea *Moina macrocopa*. In the acute exposure experiment, the 1/10 LC50 value of triclosan significantly reduced survival, feeding rate, thoracic limb activity, heart activity, and acetylcholinesterase activity. In response to the 1/10 LC50 value, intracellular reactive oxygen species increased along with elevated levels of malondialdehyde and glutathione. Enzymatic activities of catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase were significantly increased by the 1/10 LC50 value, suggesting active protection of the antioxidant defense system against oxidative stress. Chronic exposure to the 1/10 NOEC and NOEC values revealed multigenerational adverse impacts of triclosan. The second generation was found to be the most sensitive to triclosan, as the NOEC value significantly reduced the survival rate, body length, and the number of neonates per brood, along with a delayed hatching period. Taken together, these results indicate that even sublethal levels of triclosan can have detrimental effects on the water flea population's maintenance through intergenerational toxicity.

1. Introduction

Triclosan [5-chloro-2-(2,4-dichlorophenoxy)-phenol; C₁₂H₇Cl₃O₂] is a chlorinated aromatic hydrocarbon with antimycotic and antimicrobial functional groups, such as ether and phenol (Ahn et al., 2008; Dhillon et al., 2015). It finds wide usage in cosmetics (e.g., fragrances, shampoos, and moisturizers), drugs (e.g., isoniazid and diazaborine), and general health products (e.g., toothpaste, acne treatment, skin cleanser, and lotion). Due to its extensive use, facile release into the environment, and incomplete removal through wastewater treatment, triclosan is frequently detected in various environmental matrices, including effluents, surface water, drinking water, and groundwater (Halden and Paull, 2005; Ramaswamy et al., 2011). Its increasing environmental presence, coupled with its relatively strong lipophilicity

indicated by a high log K_{ow} (octanol–water partition coefficient) of 4.8, renders it easily accessible to organisms. Consequently, its bioconcentration, bioaccumulation, and evident toxicity have been consistently documented in diverse aquatic animals (Oliveira et al., 2009; Dann and Hontela, 2011). Triclosan can biomagnify through bioaccumulation from lower to higher trophic levels in aquatic food chains (Orvos et al., 2002). This bioaccumulation of triclosan can adversely affect non-target organisms in aquatic ecosystems. During its degradation and transport process, triclosan can be transformed into different derivatives such as dioxin, chloroform, 2, 4-dichlorophenol or methyl triclosan (Pycke et al., 2014; Kumar et al., 2021). These triclosan derivatives are considerably more toxic and persistent than its original form (Sacks and Lohmann, 2011; Pycke et al., 2014). The prevalence of triclosan in aquatic environments and its toxicity to aquatic organisms

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Detrimental effects of hull cleaning wastewater on oxidative status, life cycle parameters, and population growth of the monogonont rotifer *Brachionus manjavacas*

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ABSTRACT

While wastewater discharged from in-water cleaning process of ship hulls on rotifer consistently released into aquatic ecosystem, its detrimental effects on non-target animals are largely unclear. In this study, we provide evidence on detrimental effects of hull cleaning wastewater in the monogonont rotifer *Brachionus manjavacas* by analyzing biochemical and physiological parameters in its oxidative status, survival, lifespan, growth, fecundity, and population. The wastewater contained high concentrations of metals (Zn and Cu) and metal-based antifoulants (CuPT and ZnPT). Significant oxidative stress was observed in response to two wastewater samples [1) raw wastewater (RW) and 2) mechanical filtrated in the cleaning system (MF)]. Higher detrimental effects in survival, lifespan, fecundity, and population growth for 10 days were measured in the RW-exposed rotifers than those results analyzed in the MF-exposed rotifers. Two growth parameters, loric length and width were also significantly modulated by both wastewater samples. These results indicate that even filtered hull cleaning wastewater would have deleterious effects on the maintenance of the rotifer population when they exposed constantly.

1. Introduction

Biofouling is the unwanted adhesion and colonization of aquatic animals (e.g., bacteria, algae, barnacles, mussels, etc.) on surfaces of ship hulls immersed in or exposed to the aquatic environment (Bixler and Bhushan, 2012). Two major coating technologies, biocidal antifouling and non-biocidal foul release coatings have been applied to prevent biofouling process on submerged structure. Biocidal antifouling methodology contain toxic antifoulants developed to target biofilm and adhesive animals. Antifoulants practically contribute to decrease friction, fittings corrosion, and fuel consumption of the vessels by inhibition of adhesion and growth of biofouling species. Copper and metal-based booster biocides, such as copper pyrithione (CuPT) and zinc pyrithione (ZnPT), have been widely used in antifouling paints as alternatives of organotins (Yebra et al., 2004). Additional biocides or booster biocides

[e.g., chlorothalonil, 4,5-dichloro-2-n-octyl-isothiazolin-3-one (DCOIT, Sea-Nine 211), dichlofluanid, diuron, 2-(tert-butylamino)-4-(cyclopropylamino)-6-(methylthio)-1,3,5-triazine (Irgarol-1051), 2,3,5,6-tetrachloro-4-methylsulphonyl (TCMS) pyridine, thio-cyanatomethylthio-benzothiazole (TCMTB), and zineb] have been developed to be environmentally less harmful components compared to the organotins. In addition, numerous substances (e.g., booster biocides, biocides, metals, pigments, and solvents) are used in a paint mixture (Thomas et al., 2002; Soroldoni et al., 2017), while there are limited management regulations on the usage of antifouling paints.

As current antifouling technologies are not effective on the inhibition of biofilm formation and macrofouling, regular cleaning process is required to maintain vessel performance. Each component can directly be released from ship hulls and dissolved into aquatic environment. Emerging contaminants, their mixture can also be released as paint

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Hull-cleaning wastewater poses serious acute and chronic toxicity to a marine mysid—A multigenerational study

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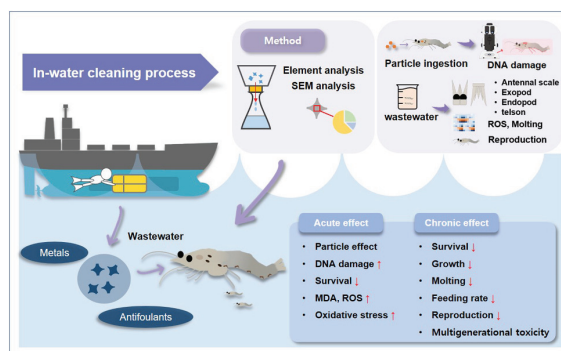
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HIGHLIGHTS

- Even filtrated wastewater contained high concentrations of metals and biocides.
- Particles suspended in the wastewater were ingested by mysids and induced DNA damage.
- Wastewater acutely increased mortality and induced oxidative stress.
- Chronic exposure to wastewater reduced feeding and growth and inhibited reproduction.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

In-water cleaning
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Multigeneration

ABSTRACT

We conducted a comprehensive assessment involving acute effects on 96-hour survival and biochemical parameters, as well as chronic effects on growth and reproduction spanning three generations of the marine mysid *Neomysis awatschensis* exposed to filtered wastewater to evaluate the potential impact of ship hull-cleaning wastewater on crustaceans. The analyzed wastewater exhibited elevated concentrations of metals, specifically zinc (Zn) and copper (Cu) and metal-based antifoulants, i.e., Cu pyriothione (CuPT) and Zn pyriothione (ZnPT). The results revealed dose-dependent reductions in survival rates, accompanied by a notable increase in oxidative stress, in response to the sublethal values of two wastewater samples: 1) mechanically filtered using the cleaning

Abbreviations: ASW, Artificial seawater; ATCh, Acetylthiocholine iodide; CAT, Catalase; CDNB, 1-chloro-2,4-dinitrobenzene; CuPT, Copper pyriothione; DCOIT, 4,5-dichloro-2-n-octyl-isothiazolin-3-one; DO, Dissolved oxygen; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); EDTA, Ethylenediaminetetraacetic acid; EDX, Energy-Dispersive X-ray; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Glutathione; Irgarol-1051, 2-(tert-butylamino)-4-(cyclopropylamino)-6-(methylthio)-1,3,5-triazine; LF, Filtration in the laboratory; MDA, Malondialdehyde; MF, Mechanical filtration in the cleaning system; PMSF, Phenylmethylsulfonyl fluoride; ROS, Reactive oxygen species; RW, Raw wastewater; SEM, Scanning electron microscope; SOD, Superoxide dismutase; TCMS, 2,3,5,6-tetrachloro-4-methylsulphonyl; TCMTB, Thiocyanatomethylthio-benzothiazole; ZnPT, Zinc pyriothione.

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Exposure to hull cleaning wastewater induces mortality through oxidative stress and cholinergic disturbance in the marine polychaete *Perinereis aibuhitensis*

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ABSTRACT

While wastewater and paint particles discharged from the in-water cleaning process of ship hulls are consistently released into benthic ecosystems, their hazardous effects on non-target animals remain largely unclear. In this study, we provide evidence on acute harmful effects of hull cleaning wastewater in marine polychaete *Perinereis aibuhitensis* by analyzing physiological and biochemical parameters such as survival, burrowing activity, and oxidative status. Raw wastewater samples were collected during ship hull cleaning processes in the field. Two wastewater samples for the exposure experiment were prepared in the laboratory: 1) mechanically filtered in the in-water cleaning system (MF) and 2) additionally filtered with a 0.45 µm filter in the laboratory (LF). These wastewater samples contained high concentrations of metals (zinc and copper) and metal-based booster biocides (copper pyrithione and zinc pyrithione) compared to those analyzed in seawater. Polychaetes were exposed to different concentrations of the two wastewater samples for 96 h. Higher mortality was observed in response to MF compared to LF-exposed polychaetes. Both wastewater samples dose-dependently decreased burrowing activity and AChE activity. Drastic oxidative stress was observed in response to the two wastewater samples. MDA levels were significantly increased by MF and LF samples. Significant GSH depletion was observed with MF exposure, while increased and decreased GSH contents were observed in LF-exposed polychaetes. Enzymatic activities of antioxidant components, catalase, superoxide dismutase, and glutathione S-transferase were significantly modulated by both wastewater samples. These results indicate that even filtered hull cleaning wastewater can have deleterious effects on the health status of polychaetes.

1. Introduction

Numerous aquatic organisms adhere and accumulate on the surfaces of ship hulls submerged or exposed to the aquatic environment, leading to undesired biofouling (Bixler and Bhushan, 2012). The complex and dynamic process of biofouling generally involves dynamic shifts from thin biofilm formation to accumulation of macrofouling communities (Abarzua and Jakubowski, 1995; Abed et al., 2019). Biofouling causes

significant economic loss and negative impact on hydrodynamic performance of the vessels through increase in fuel consumption and exhaust emission due to water resistance and increased weight (Schultz et al., 2011; Lindholdt et al., 2015). Huge biofouling directly increases maintenance costs and hydrodynamic resistance with decreases in flow and efficiency of internal seawater systems of the vessels (Schultz et al., 2011; Lindholdt et al., 2015). It also can lead to the transport of invasive alien species by shipping and damage local and endemic biodiversity

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Chronic toxic effects of fragmented polyvinyl chloride on the water flea *Moina macrocopa*

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Abstract

Background Although many studies have reported the detrimental potential of commercially synthesized sphere types of microplastics (MPs) and nanoplastics (NPs) on aquatic ecosystems, limited information is available on fragmented MPs and their chronic effects.

Objective This study aimed to evaluate potentially harmful effects of fragmented polyvinyl chloride (PVC; < 20 µm) in a range from 0.001 to 500 µg L⁻¹ on a water flea model, *Moina macrocopa*. Physiological and biochemical effects of fragmented PVC were studied by analyzing the mortality, growth, and reproduction parameters, with evidence retrieved from biochemical components for 14 days.

Results Waterborne exposure to PVC fragments resulted in temporal bioconcentration through ingestion. The PVC fragments were egested in feces. The mortality was drastically increased by relatively higher concentrations (> 10 µg L⁻¹) of fragmented PVC compared to that of the control group. Two concentrations of PVC fragments (100 and 500 µg L⁻¹) significantly induced growth retardation, with expansion of intermolt periods and decreased the first day of reproduction and the number of neonates per brood. Exposure to PVC fragments induced fluctuations in oxidative status and antioxidant components, with inhibition in acetylcholinesterase activity.

Conclusion These results imply that fragmentation of MPs can induce a significant physiological and biochemical effects to aquatic crustaceans by inducing strong oxidative stress and impairing antioxidant defense.

Keywords Water flea · Fragmented microplastic · Polyvinyl chloride · Life-cycle parameter · Antioxidant defense

Introduction

Plastic pollution has rapidly emerged as one of the significant environmental issues due to its abundance in the aquatic ecosystem (Geyer et al. 2017). Plastic production has exponentially expanded since the 1950s, resulting in the direct and indirect discharge of millions of tons of plastic waste into aquatic environments. In freshwater ecosystems, the majority of plastic litter is derived from waste management, accidental or intentional discharges and dumping, and anthropogenic activities (e.g., manufacturing, farming, household consumption, and recreation) (Alimi et al. 2018). However, little is known about the emission rates of plastic debris into aquatic systems. Plastic debris tends to accumulate in aquatic systems due to its slow rate of biodegradation (Lebreton et al. 2018). After being released into the aquatic environment, plastics can be fragmented and degraded into much smaller microplastics (MPs) and nanoplastics (NPs) by numerous mechanical and photochemical forces (Lambert

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Research article

Acute and chronic effects of the antifouling booster biocide Irgarol 1051 on the water flea *Moina macrocopa* revealed by multi-biomarker determination

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Oxidative stress

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ABSTRACT

Irgarol 1051 is an herbicide extensively utilized in antifouling paint due to its ability to inhibit photosynthesis. Irgarol and its photodegradation products are highly persistent in waters and sediments, although they are present in low concentrations. However, our understanding of the harmful effects of Irgarol on non-target organisms remains limited. In this study, we assessed the effects of acute (24 h) and chronic (14 days across three generations) exposure to different concentrations (including the 1/10 NOEC, NOEC, and 1/10 LC50 calculated from the 24-h acute toxicity test) of Irgarol using the water flea *Moina macrocopa*. Acute exposure to 1/10 LC50 significantly decreased survival, feeding rate, thoracic limb activity, heart rate, and acetylcholinesterase activity. Elevated levels of intracellular reactive oxygen species and malondialdehyde, along with a significant increase in catalase and superoxide dismutase activity, suggested the induction of oxidative stress in response to 1/10 LC50. An initial boost in glutathione level and the enzymatic activities of glutathione peroxidase and glutathione reductase, followed by a plunge, implies some compromise in the antioxidant defense system. Upon chronic exposure to the NOEC value, both generations F1 and F2 displayed a significant decrease in survival rate, body length, number of neonates per brood, and delayed sexual maturation, suggesting maternal transfer of potential damage through generations. Taken together, Irgarol induced acute toxicity through physiological and cholinergic damage, accompanied by the induction of oxidative stress, in the water flea. Even its sub-lethal concentrations can induce detrimental effects across generations when consistently exposed.

1. Introduction

Irgarol 1051 [also known as cybutryne; 2-(ter-butylamino)-4-(cyclopropylamino)-6-(methylthio)-1,3,5-triazine; hereafter referred to as Irgarol] as an algicide has been utilized in antifouling coatings and preservatives utilized in shipbuilding materials, such as exterior paints, stucco, caulks, vinyl roofing, wood protective stains, and joint cement (Hall Jr. et al., 1999). It is frequently combined with copper compounds to enhance its effectiveness in protecting against biofouling. Because of its extensive use, low water solubility ($< 7 \text{ mg L}^{-1}$), and relatively long half-life spans from 100 to 350 days (Steen et al., 1997; Hall Jr. et al., 1999; Scarlett et al., 1999; Thomas et al., 2002; Zhou, 2008), Irgarol has been consistently detected worldwide in diverse aquatic environments, including sediments. The environmental risk limit (ERL) for Irgarol is set

at 24 ng L^{-1} for water and $1.4 \text{ } \mu\text{g Kg}^{-1}$ for soil and sediment (van Wezel and van Vlaardingen, 2004). However, higher levels of Irgarol exceeding its ERL value have been documented in several regions. For instance, in the East Anglia in the United Kingdom, concentrations of Irgarol ranged from 1 to 1250 ng L^{-1} (Lambert et al., 2006). Similarly, in the Lake Geneva in Switzerland and France, its levels ranged from 10 to 135 ng L^{-1} (Nyström et al., 2002). In the Bay of Vilaine area, Brittany and France, its concentrations were measured from 110 to 710 ng L^{-1} (Caquet et al., 2013).

Irgarol was initially developed to target autotrophs like algae and cyanobacteria by substituting plastoquinone and inhibiting the QB-binding site of the D1 protein in photosystem II (PS II) (Dahl and Blanck, 1996; Okamura et al., 2000). This action impedes electron transport, leading to a subsequent reduction in ATP and NADPH^{+}

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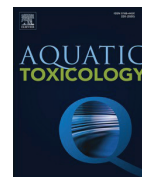
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Negligible additive effect of environmental concentrations of fragmented polyethylene terephthalate microplastics on the growth and reproductive performance of Java medaka exposed to 17 β -estradiol and bisphenol A

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Fragmented microplastic
Endocrine disrupting chemical
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Vitellogenin

ABSTRACT

To investigate whether environmental concentrations of fragmented polyethylene terephthalate (PET) microplastics (MPs) have additional or combined effects on endocrine-disrupting activity, Java medaka (*Oryzias latipes*) were exposed to 17 β -estradiol (E2; 5, 10, 50, and 100 ng L⁻¹), bisphenol A (BPA; 5, 10, 50, and 100 μ g L⁻¹), and E2 and BPA combined with PET MPs (1 and 100 particles L⁻¹) for 200 days. The growth parameters, such as body length and weight, were significantly decreased by the highest concentrations of E2 and BPA. A significant reduction in egg production was observed in female fish exposed to BPA, with an additive toxic effect of PET MPs. A female-biased sex ratio was observed in fish exposed to both chemicals. Exposure to E2 significantly increased the hepatosomatic index (HSI) in both sexes, while no significant effect was observed in the gonadosomatic index (GSI). Exposure to BPA significantly increased the HSI in female fish and decreased the GSI in both sexes of fish. An additive effect of PET MPs was observed on the GSI value of female exposed to BPA. Significant elevations in vitellogenin (VTG) levels were observed in both sexes due to exposure to E2 and BPA. Additive effects of PET MPs were observed on VTG levels in males exposed to E2 and BPA. Taken together, even long-term treatment with PET MPs induced only a negligible additive effect on the endocrine-disrupting activity in Java medaka at environmentally relevant concentrations.

1. Introduction

Plastic pollution is one of the emerging and severe global issues, specifically in whole aquatic environments, and it has garnered international attention (Van Sebille et al., 2020). Previous reports have indicated an increasing trend in the western North Atlantic from 1986 to 2015, with a rate of increase, doubling approximately every 11 years, that currently mirrors global cumulative plastic production (Eriksen et al., 2023; Wilcox et al., 2019). In aquatic systems, various plastics, including bottles, plastic bags, and fishing nets, gradually fragment into microplastics (MPs) due to exposure to physical factors such as waves, heat, or ultraviolet radiation, as well as biological degradation (Xu et al., 2020). The global mass of plastic is currently estimated to be composed of 170 trillion plastic pieces in marine environments (Eriksen et al.,

2023). Although the abundance of MPs can vary due to numerous environmental factors, it has been reported that there is an estimated average of 0.03 microplastics per m³ floating on the surface of oceans (Bohdan, 2022). Environmental concentrations of MPs vary widely in the oceans. In particular, in East to Southeast Asia, where is the habitat area of the Java medaka used in this study, the abundance of MPs was reported to be 0.49 particles L⁻¹ in the northern coastal waters of Surabaya, Indonesia (Cordova et al., 2019), 106–238 and 143–196 particles mL⁻¹ in the Johor and Singapore Straits (Curren and Leong, 2023), and 2.1–6.8 particles L⁻¹ in coastal and estuarine zones of Malaysia (Zainuddin et al., 2022). Among MPs, PETs are crucial materials for disposable bottles and cans. However, PET accumulation has become a major issue of plastic debris in the oceans (Kim et al., 2020; Paladhi et al., 2022). In the area of the Java medaka habitat, the abundance of

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Article

Annotation and Characterization of the *Zacco platypus* Genome

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Abstract: The pale chub *Zacco platypus* (Cypriniformes; Xenocyprididae; Jordan & Evermann, 1902) is widely distributed across freshwater ecosystems in East Asia and has been recognized as a potential model fish species for ecotoxicology and environmental monitoring. Here, a high-quality de novo genome assembly of *Z. platypus* was constructed through the integration of a combination of long-read Pacific Bioscience (PacBio) sequencing, short-read Illumina sequencing, and Hi-C sequencing technologies. *Z. platypus* has the smallest genome size compared to other species belonging to the order Cypriniformes. The assembled genome encompasses 41.45% repeat sequences. As shown in other fish, a positive correlation was observed between genome size and the composition of transposable elements (TE) in the genome. Among TEs, a relatively higher rate of DNA transposon was observed, which is a common pattern in the members of the order Cypriniformes. Functional annotation was processed using four representative databases, identifying a core set of 12,907 genes shared among them. Orthologous gene family analysis revealed that *Z. platypus* has experienced more gene family contraction rather than expansion compared to other Cypriniformes species. Among the uniquely expanded gene families in *Z. platypus*, detoxification and stress-related gene families were identified, suggesting that this species could represent a promising model for ecotoxicology and environmental monitoring. Taken together, the *Z. platypus* genome assembly will provide valuable data for omics-based health assessments in aquatic ecosystems, offering further insights into the environmental and ecological facets within this species.

Keywords: Xenocyprididae; fish genome; *Zacco platypus*; de novo genome assembly



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1. Introduction

As fish are diverse worldwide, they can be used to evaluate pollutant effects in local regions and to understand the mechanisms of action underlying unknown toxicity in the field [1,2]. In current ecotoxicological research, especially in freshwater environments, model fish such as zebrafish and Japanese medaka have been extensively utilized [3–5]. However, local and sentinel fish species have been used sparingly. The model organisms are not indigenous species, and laboratory experiments rarely reflect natural or real-world settings. Consequently, it is difficult to directly apply the results obtained from model animals to the health and risk assessment of domestic aquatic ecosystems. Freshwater environments globally are threatened by numerous anthropogenic, abiotic, and biotic challenges, including direct or indirect disposals and runoff from land [6,7]. Monitoring and risk assessments of aquatic ecosystems' health using sentinel fish are crucial to understanding the actual safe and habitable status of these environments [8]. Model fish cannot fully represent the actual environmental status, and chemical characterization of pollutants in water bodies can provide only partial information about ecosystem responses.

The pale chub, *Zacco platypus*, is one of the indicator species used for assessing water quality [9]. This fish can be easily raised in artificial culture conditions in the laboratory,

Article

The Effects of Co-Exposure to Antifoulants and Microplastics on the Survival, Oxidative Status, and Cholinergic System of a Marine Mysid

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Abstract: Antifoulants such as copper pyrithione (CuPT) and zinc pyrithione (ZnPT) are widespread and hazardous pollutants in aquatic environments. The presence of microplastics (MPs) introduces significant uncertainty regarding the toxicity of CuPT and ZnPT, as their effects can be influenced by MPs. There is a limited understanding of the toxic potential of CuPT and ZnPT when they coexist with MPs. Here, the marine mysid *Neomysis avatchensis* was treated using no observed effect concentration (NOEC) values of CuPT and ZnPT premixed with MPs (1 μm ; 1–100 particles mL^{-1}). The presence of MPs increased the toxicity of the antifoulants in juvenile and adult mysids over 96 h. The additive effect of the MPs varied by chemical; feeding was only reduced by CuPT with MPs, whereas no fluctuation in feeding was observed in response to ZnPT with MPs. Co-exposure to antifoulants and MPs increased malonaldehyde levels, but the response of antioxidant components varied by chemical. In mysids co-exposed to CuPT and MPs, the activity levels of catalase and superoxide dismutase were decreased, whereas their enzymatic activity levels were elevated by co-exposure to ZnPT and MPs. Similarly, depletion of glutathione (GSH) was observed in mysids co-exposed to CuPT and MPs, with significant reductions in GSH reductase (GR) and peroxidase (GPx). However, the GSH level was increased by co-exposure to ZnPT and MPs, with elevations in GR and GPx activity levels. Significant inhibition of acetylcholinesterase activity was only observed in response to CuPT and MPs. These results suggest that MPs can increase toxicity via additive and/or synergistic effects through oxidative imbalance, but these effects of MPs can vary with different chemicals.

Keywords: marine mysid; metal-based antifoulant; microplastic; oxidative stress; antioxidant response



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1. Introduction

Biofouling refers to the colonization of diverse aquatic animals such as bacteria, algae, barnacles, and mussels on submerged surfaces like ship hulls, artificial structures in mariculture, and floating platforms. This phenomenon leads to substantial economic losses and environmental impacts [1]. Antifouling paints and coatings incorporating copper pyrithione (CuPT) and zinc pyrithione (ZnPT) have been extensively used to protect the natural and artificial surfaces of ships and aquatic structures against fouling organisms (e.g., bacteria, microalgae, invertebrates), acting as potential replacements for organotin compounds, which were banned due to their significant toxicity to non-target organisms [2,3]. Due to the higher efficiency of metal-based antifouling agents, resulting from low water solubility, broad bactericidal, algicidal, fungicidal, and antimicrobial actions, and favorable environmental chemistry for numerous applications, the use of CuPT and ZnPT has expanded dramatically worldwide [4]. While environmental concentrations of ZnPT have been predicted to reach



Unlocking the relationship between tourism and bivalve health: A comprehensive study of *Donax trunculus* as environmental and public health indicators in Morocco

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ABSTRACT

This study assessed the levels of Cd, Cu and Pb in the sentinel bivalve *Donax trunculus* collected from two touristic Moroccan sandy beaches, namely Agadir and Taghazout, in Morocco. The results revealed higher metal contamination in *D. trunculus* specimens collected from Taghazout compared to those from Agadir. Among the metals analyzed, Pb was found to pose the greatest risk to human health, followed by Cd and Cu. The study also investigated the presence of the trematode parasite *Bacciger bacciger* in *D. trunculus* specimens. The parasite was found only in female *D. trunculus* from Taghazout, with infection rates ranging from 11% to 46%. Furthermore, significantly higher levels of oxidative stress biomarkers were observed in specimens from Taghazout, where positive correlations were observed between Cu, Cd, Glutathione S-Transferase, and parasite prevalence, as well as between Pb and Catalase. In the case of *D. trunculus* collected from Agadir, Malondialdehyde levels were positively correlated with Cd and negatively correlated with Pb, while Catalase levels were negatively correlated with Cu. Complex interactions emerged between metal pollution, parasitic infection, and oxidative stress biomarkers in *D. trunculus* from two Moroccan coastal sites. Evaluating biotic stress synergies with biomarkers shows promise for integrating biological implications of coastal contamination.

1. Introduction

Coastal tourism is a major socioeconomic activity across the world that has seen considerable growth globally in recent decades. According to the OECD report (2016), it is expected that by 2030, tourism will become the world's largest economic sector, generating \$777 billion in global revenues and providing 8.6 million jobs. However, if not properly managed, anthropogenic activities associated with coastal tourism, such as the improper disposal of residential waste, touristic activities, and fisheries, can have a detrimental impact on marine and coastal ecosystems, thereby compromising their environmental health (Cancaccapa

et al., 2016; Furlan et al., 2019; Lazzari et al., 2021; Lu et al., 2018; Sadutto et al., 2021). This negative impact is often exacerbated during periods of high tourist influx, such as summer seasons, festive periods, holidays, and fluctuations in weather conditions (Corluka et al., 2016; Mghili et al., 2023). These periods typically witness increased human activities and pollution, leading to elevated hazardous pressures on the aquatic animals. Thus, it is crucial to recognize the potential consequences of inadequate management in touristic beaches and take proactive measures to mitigate the negative effects on marine and coastal ecosystems.

The Moroccan tourism sector has undergone significant changes over

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journal homepage: www.elsevier.com/locate/cbpcAcute and multigenerational effects of short-chain chlorinated paraffins on the harpacticoid copepod *Tigriopus japonicus*Jihyun Lee^a, Seong Duk Do^a, Jae-Sung Rhee^{a,b,c,*}^a Department of Marine Science, College of Natural Sciences, Incheon National University, Incheon 22012, Republic of Korea^b Research Institute of Basic Sciences, Core Research Institute, Incheon National University, Incheon 22012, Republic of Korea^c Yellow Sea Research Institute, Incheon 22012, Republic of Korea

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ABSTRACT

Although the measurement of short-chain chlorinated paraffins (SCCPs) in aquatic ecosystems has increased, limited information is available on their toxic effects on aquatic animals. To evaluate the harmful effects of SCCPs, we assessed their acute impact on 24-h survival and biochemical parameters, as well as their chronic effects on growth and reproduction over three generations in the harpacticoid copepod *Tigriopus japonicus*. Dose-dependent increases in mortality were observed, with an LC50 value of 74.6 $\mu\text{g L}^{-1}$ for 24 h. Acute exposure to the LC10 value for 24 h significantly reduced feeding behavior, accompanied by a notable decrease in acetylcholinesterase enzymatic activity. Simultaneously, the intracellular levels of reactive oxygen species increased, along with elevated malondialdehyde contents. Glutathione level was increased by the LC10 value of SCCPs with the induction of enzymatic activities of antioxidant defense components, including glutathione S-transferase, catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase. When *T. japonicus* was continuously exposed to 1/10 of the NOEC and NOEC values for 12 days across three generations (F0–F2), growth retardation was observed in the F2 generation, with delay in the developmental periods from nauplius to adult. Although the total number of nauplii per brood was not significantly altered across generations, a significant delay in the onset of reproduction was observed in the F2 generation. Our findings suggest that even sublethal concentrations of SCCPs can negatively affect the health of copepod populations with consistent exposure.

1. Introduction

Chlorinated paraffins (CPs; also known as polychlorinated *n*-alkanes (PCAs; $\text{C}_n\text{H}_{2n+2-2x}\text{Cl}_x$) are emerging as a class of persistent organic pollutants (POPs) of global concern (Bayen et al., 2006; EPA, 2009). CPs are mixtures composed of C10 to C30 *n*-alkanes with chlorine content ranging from 30 % to 70 % by mass, encompassing an extensive array of approximately 8.9×10^7 varieties (Yuan et al., 2019; Vetter et al., 2022). They are classified based on their carbon atoms into short-chain CPs (C10–13, SCCPs), medium-chain CPs (C14–C17, MCCPs), and long-chain CPs ($\geq\text{C18}$, LCCPs) (van Mourik et al., 2018). Based on their chain length and chlorination rate, CPs have a wide range of viscosity, density, and volatility (McGrath et al., 2022). CPs are characterized by their chemical stability, persistence, ability to bioaccumulate, and propensity for long-range transport from pole to pole (van Mourik et al., 2018). Moreover, at high temperatures, CPs release hydrochloric acid and

exhibit poor combustion as the chlorine content increases (Mukherjee, 1990). Because of these chemical properties, CPs have found widespread use across various industries since the 1930s (van Mourik et al., 2016). They serve as plasticizers, metalworking fluids, flame retardants, lubricants, and additives in paints, adhesives, textiles, sealants, rubber, and printing inks (van Mourik et al., 2018; Chen et al., 2022). According to the global dynamic substance flow analysis model, the worldwide production of CPs amounted to 33 million metric tons, and cumulative emissions had reached 5.2 million metric tons by 2020 (Chen et al., 2022).

The short-chain CPs, SCCPs have been detected in environmental samples and organisms throughout the world (van Mourik et al., 2016; Nevondo and Okonkwo, 2021; Lyu and Zhang, 2023). Although predominantly measured in China, environmental concentrations of SCCPs have been reported in several regions, ranging from nanogram to microgram levels (Ma et al., 2014, 2018; Huang et al., 2019; Zhao et al.,

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Detrimental effects of hull-cleaning wastewater on embryo development via oxidative stress induction in the Java medaka *Oryzias javanicus*

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Abstract

Objective Considerable amounts of paint particles and debris are released into coastal areas during the physical cleaning of ship hulls. However, there is a paucity of data regarding their potential toxic impacts on non-target aquatic organisms. Therefore, we undertook a thorough evaluation encompassing acute survival effects and developmental parameters of Java medaka *Oryzias javanicus* embryos exposed to filtered wastewater, alongside the assessment of alterations in antioxidant constituents.

Methods Embryos were subjected to different concentrations of filtered wastewater (up to 1%) subsequent to fertilization. Analysis encompassed the examination of embryo and hatched larvae survival rates, time taken for hatching, and developmental parameters, coupled with biochemical assessments of oxidative stress and DNA damage markers.

Results Dose-dependent mortality, hatching delays, and developmental abnormalities were observed in embryos. Concurrently, there were notable increases in intracellular reactive oxygen species and malondialdehyde levels, accompanied by elevated enzymatic activities of glutathione *S*-transferase, glutathione peroxidase, catalase, and superoxide dismutase. In addition, DNA damage was observed at the highest concentration (1%) of wastewater treated in this study.

Conclusions The observed changes in in vivo parameters and biochemical constituents indicate that sublethal concentrations of hull-cleaning wastewater exerted a toxic influence on Java medaka embryos.

Keywords Hull-cleaning wastewater · Java medaka · Growth parameters · Oxidative stress · DNA damage

Introduction

Biofouling refers to the undesirable attachment and colonization of various aquatic organisms (such as bacteria, algae, barnacles, mussels, etc.) on surfaces of ship hulls that are submerged in or exposed to the aquatic environment [1]. The buildup of biofouling on submerged surfaces of ship hulls leads to significant economic losses and adversely

impacts the hydrodynamic performance of vessels [2, 3]. Various substances, encompassing metals, biocides, booster biocides, pigments, solvents, and their combinations, have been widely employed as antifoulants in antifouling paints to deter biofouling on ship surfaces [4–6]. Antifoulants are pivotal in mitigating friction, averting corrosion of fittings, and reducing fuel consumption in vessels by impeding the settlement of marine organisms on the ship's surfaces. Nevertheless, these compounds are acknowledged as significant contributors to aquatic pollution due to their persistency in both water column and sediments, and their potential detrimental effects on numerous aquatic organisms have been consistently documented, prompting public concern.

Since current antifouling technologies are ineffective at inhibiting biofilm formation and macrofouling, regular cleaning processes are necessary to uphold vessel performance. Almost antifoulant component has the potential to leach naturally from ship hulls into the aquatic environment. Alternatively, a combination of these substances may

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Exposure to acute cold temperatures induces Ucps-dependent thermogenesis in zebrafish

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Abstract

Background Cold adaptation is a vital mechanism for maintaining homeostasis in both fish and warm-blooded animals. One of the key components involved in this process is uncoupling protein 1 (Ucp1), which is expressed in brown adipose tissue (BAT) or beige adipose tissue (BeAT) when exposed to low temperatures. Ucp1 is a mitochondrial transport protein that, along with other Ucp families such as Ucp2 and Ucp3, plays an important role in non-shivering thermogenesis (NST) in BAT or BeAT. Despite the importance of cold adaptation in fish, our current understanding of the mechanisms underlying this process is incomplete and warrants further investigation.

Objective To understand the potential biochemical role of Ucps in thermoregulation and cold-water adaptation in fish, we analyzed mRNA expressions of Ucp families and key brown adipogenic markers involved in Ucp1-dependent thermogenesis (i.e., *prdm16* and *cidea*) in zebrafish (*Danio rerio*) that were exposed to relatively low temperature (18 °C) in comparison to the control group maintained at 28 °C.

Methods To compare the homology of Ucps sequences between zebrafish and mammals, we conducted sequence alignment using T-coffee multiple sequence alignment (ver. 11.00) with pair-wise comparisons and calculated the similarities and identities between sequences. To analyze the time course pattern of cold exposure, zebrafish were exposed to two temperatures of 28 °C and 18 °C for 3, 6, 12, and 24 hours, respectively. Cold-induced adipogenesis was performed quantitative real-time PCR and gene expression was calculated using the 2- $\Delta\Delta C_t$ method.

Results The mRNA expressions of *ucp1* gene showed time-dependent increases in the group exposed to 18 °C when compared to the control group. However, the mRNA expressions of *ucp2* and *ucp3* exhibited different modulation patterns over the time-course of the experiment. Furthermore, the mRNA expression levels of genes associated with adipogenesis that typically occurs in brown adipocytes (i.e., *prdm16* and *cidea*) were significantly elevated following exposure to 18 °C in a time-course dependent manner.

Conclusion Following exposure to acute low temperature, the expression of Ucp1 was noticeably increased at the transcriptional level. Concurrently, the mRNA expressions of other Ucp family members such as *ucp2-5* as well as brown adipogenic markers were also changed. This suggests that there may be a Ucps-dependent NST mechanism involved in the adaptation of zebrafish, a cold-blooded animal, to low temperature.

Keywords Cold adaptation · Thermogenesis · Adipogenesis · Ucps · Zebrafish

Young-Joo Yun and Sang-Eun Nam equally contributed to this work.

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Introduction

Mammals, including humans, are warm-blooded animals and must maintain a stable core body temperature. Hyperthermia, defined as core body temperature above 40.5 °C, can be dangerous, leading to conditions such as seizures and coma. On the other hand, hypothermia, defined as a core body temperature below 35.0 °C, can also be severe and result in respiratory depression, cardiac arrhythmias, mental dysfunction, heart failure, and coma and death, occurring twice as frequently as death from hyperthermia



Assessing photosynthetic uptake of total inorganic carbon in an *Ecklonia cava* dominated seaweed artificial reef: Population- and community-level metabolisms

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Abstract

With increasing climate crisis concerns, interest in primary production of marine ecosystems is also increasing. Recently, artificial transplantation of canopy-forming seaweed has been carried out to increase coastal productivity, but the contribution of this strategy to inorganic carbon uptake has not been evaluated. Here, we estimated photosynthetic uptake of total inorganic carbon (TIC) by measuring the population and community productivity of artificial reefs (ARs) implanted with seaweed (dominated by *Ecklonia cava*) to determine TIC uptake efficiency of the transplanted seaweed and ecosystem respiration. The community-level of TIC uptake rate in the ARs ecosystem was measured using a 24-h tent incubation method in July and October 2021 in the East Sea of Korea. From these experiments, GCP and NCP (gross and net community production, respectively) ranged from 0.81–4.84 g C m⁻² day⁻¹ and from -2.08–1.91 g C m⁻² day⁻¹, respectively. We also measured photosynthesis-irradiance curves of *E. cava* (population level) to estimate individual inorganic carbon uptake rate in October 2021. GPP and NPP (gross and net primary productivity) were 132.47 and 6.83 mg C ind.⁻¹ day⁻¹, respectively. Based on these results, we estimated TIC uptake rates via photosynthesis of ARs seaweed beds at a community level. About 95% of GCP was allocated for *E. cava* respiration, but seaweed-associated respiration at different trophic levels (i.e., microbial and meiofauna/macrofauna respiration) was allocated an additional 20%, creating a net heterotrophic environment. Together, productivity differences at the population and community levels are key in explaining seaweed contribution to TIC uptake and respiration at the community level.

Keywords Artificial kelp reef · Community production · Photosynthetic TIC uptake · Seaweed-associated metabolism · Seaweed carbon pool

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Review article

Harnessing the power of eDNA technology for macroalgal ecological studies: Recent advances, challenges, and future perspectives

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ABSTRACT

Macroalgae are critical to oceanic ecosystems as they help maintain ecological balance and contribute to climate regulation. Therefore, preserving and managing macroalgal biodiversity is essential to sustain the overall health of marine environments. However, ecological monitoring of macroalgal communities can be challenging owing to difficulties associated with sampling and morphological identification. Development of environmental DNA (eDNA) technology has eased sample collection and enabled analysis of species composition in mixed DNA samples from any source. In recent years, eDNA technology has played an indispensable role in advancing our understanding of macroalgal ecology. This review aims to provide an overview of the principles, basic technical process, recent developments, and practical applications of eDNA technology in macroalgal research. Besides, the problems and limitations encountered in macroalgal eDNA research are addressed, and the potential solutions to these issues are proposed. Analysis of the current research hotspots and innovative trends suggests that eDNA technology might play an increasingly important role in macroalgal research, particularly in revealing patterns of macroalgal biodiversity and assessing their contributions to carbon sequestration. This review will provide a scientific basis and technical support for the conservation and management of macroalgal ecosystems based on eDNA technology.

1. Introduction

Macroalgae, the larger species of algae that are visible to the naked eye, including brown, green, and red algae, thrive in coastal areas worldwide, covering an area of 4 million km² [1–3]. These highly productive ecosystems are responsible for a global net primary production of 2 Pg C each year [4]. Despite occupying <2 % of the seafloor, macroalgae play a crucial role in carbon sequestration, accounting for 50 % of all carbon buried in marine sediments [5]. Therefore, monitoring the distribution and movement of macroalgae is essential for comprehending their ecological function and benefits [6].

Environmental DNA (eDNA) technology provides a novel approach for identifying macroalgae in environmental samples. eDNA refers to the ubiquitous, free DNA molecules released by organisms in the environment [7], or the entire genome of a cell of free microbial and multicellular organisms in the environment [8]. The production of eDNA is

affected by an organism's biomass, age, feeding activity, physiology, life history, and overall space use [8,9]. The eDNA technique allows for non-invasive sampling of the environment for genetic testing of animal hairs, shed cells, feces, and reproductive cells in soil, sediment, water bodies, or air. When compared with traditional direct sampling, this method is less expensive and the sample collection process is less affected by environmental conditions, allowing for the collection of a larger number of samples and an improved resolution of taxonomic classification [10].

eDNA metabarcoding combines DNA barcoding and next-generation sequencing (NGS) technologies. In this approach, NGS is used to perform high-throughput sequencing of DNA fragments that have been amplified with specific primers. The raw sequences obtained are then subjected to quality control to obtain manipulable operational taxonomic units, which can be analyzed based on sequence similarity using tools such as VSEARCH and Mothur, or based on data modeling analysis using Crop, DADA2, MED, and other similar software. The obtained results are

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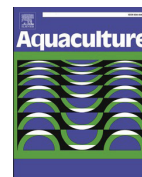
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Review

Acid treatment for the prevention of green macroalgae attachment to *Neopyropia* aquaculture

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ABSTRACT

The *Neopyropia* aquaculture area located in Subei Shoal has garnered considerable attention due to the green tide in the Yellow Sea. Acid treatment is widely used in *Neopyropia* culture, both in the seedling and mature stages. In light of this, we propose the implementation of acid treatment technology in the *Neopyropia* aquaculture area within Subei Shoal, with the primary aim of mitigating the proliferation of green macroalgae on the rafts. This, in turn, is anticipated to elevate the yield and overall quality of *Neopyropia*. In this paper, we provide a comprehensive overview of the application of acid treatment technology in *Neopyropia* aquaculture, elucidating the key acid solutions used and the underlying mechanisms governing acid treatment. Furthermore, we delve into early prevention strategies for countering the green tide within the *Neopyropia* aquaculture region of northern Jiangsu Province, ultimately striving to augment the quality of *Neopyropia* cultivation and propose effective measures for curtailing the scale of green tide.

1. Introduction

The Earth, known as the blue planet, boasts abundant seaweed resources (Kambey et al., 2021). The global seaweed cultivation production tonnage has skyrocketed over the past 70 years, from 34,700 tons to 35.1 million tons, with the production of red algae (especially *Neopyropia*) in Asia continually increasing (Cai, 2021; FAO, 2022). In China's seaweed industry, the *Neopyropia* aquaculture industry has grown to become second only to kelp farming. Currently, the main breeding varieties of *Neopyropia* in China are *Neopyropia haitanensis* and *Neopyropia yezoensis* (Wang et al., 2020a). *N. yezoensis* is the primary the predominant cultivated species of algae in Jiangsu Province, where the cultivation structure mainly consists of cables, nets, bamboo poles, foam floats, and other materials. Green macroalgae attach to the facilities, with fractured algal segments drifting into the sea, providing the initial biomass for the green tide in the Southern Yellow Sea (SYS) (See Fig. 1) (Liu et al., 2009; Keesing et al., 2011; Zhang et al., 2014; Fan et al., 2015; Song et al., 2015; Wang et al., 2018; Tang et al., 2021; Liu et al., 2021a; Li et al., 2022a).

The proliferation of green tide represents a significant ecological

challenge faced by numerous nations across the globe (Fletcher, 1996; Frankenstein, 2000; Gower and King, 2011; Liu et al., 2021a). This predicament has engaged the attention of researchers and environmentalists for an extended duration, with a definitive resolution remaining elusive. Remarkably, the Southern Yellow Sea in China has been incessantly afflicted by a persistent green tide for the past 17 years. Long-term satellite observations (Liu et al., 2013; Hu et al., 2017), floating trajectory analysis (Liu et al., 2009; Keesing et al., 2011), molecular biological identification (Zhang et al., 2014) and continuous field tracking observations during the early occurrence and development of green tides (Fan et al., 2012) have revealed that the main source of the green tide in the Yellow Sea is the green macroalgae that fall from the *Neopyropia* aquaculture rafts in Subei Shoal (See Fig. 2) (Fan et al., 2015; Song et al., 2015; Wang et al., 2018; Tang et al., 2021; Li et al., 2022a).

Acid treatment plays a pivotal role in *Neopyropia* cultivation, addressing issues related to diseases and algae epiphytation during the seedling and culture phases (Kim et al., 2014; Ward et al., 2020; Tang et al., 2021). Various methods are typically employed, such as air-exposure, seeding net refrigeration technology, freshwater treatment,

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Research Article

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Effects of biostimulants, AMPEP and Kelpak on the growth and asexual reproduction of *Pyropia yezoensis* (Bangiales, Rhodophyta) at different temperatures

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Acadian marine plant extract powder (AMPEP) and Kelpak are commercial biostimulants derived from brown algae *Ascophyllum nodosum*. This study was to determine if AMPEP and Kelpak can induce thermal resistance in *Pyropia yezoensis*. *P. yezoensis* blades were exposed to different concentrations (control: 0, low: 0.001, high: 1 ppm) of AMPEP and Kelpak at 10°C for 6 and 7 days, respectively. Those blades were then cultivated in von Stosch enriched seawater medium at different temperatures (10, 15, 20, and 25°C) with 12 : 12 L : D photoperiod and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation for additional 15 days. Results showed that *P. yezoensis* reproduced archeospores at 20 and 25°C at all biostimulant conditions within 15 days. At lower temperatures (10 and 15°C), only AMPEP-treated *P. yezoensis* reproduced archeospores. *P. yezoensis* exposed to 1 ppm Kelpak exhibited higher phycoerythrin and phycocyanin contents than control and 0.001 ppm conditions at 15°C. AMPEP-treated conditions showed higher phycoerythrin and phycocyanin contents than control at 10°C. These results suggest that AMPEP and Kelpak may not enhance the thermal resistance of *P. yezoensis*. However, AMPEP stimulated archeospores release at lower temperatures. The treatment of AMPEP and Kelpak also increased the pigment contents in *P. yezoensis*. These results suggest that the use of seaweed-derived biostimulants can provide some economic benefits in *P. yezoensis* aquaculture. The enhancement of archeospores formation by AMPEP at lower temperature may also increase the productivity since *Pyropia* farming relies on the accumulation of secondary seedlings via asexual reproduction.

Keywords: AMPEP; Archeospores; asexual reproduction; biostimulant; Kelpak; *Pyropia*; temperature

INTRODUCTION

Seaweed-derived biostimulants are mainly used in agriculture for an advance of the development of root capacities to improve nutrient use efficiency (Rayorath et al. 2008), to enhance resistance to abiotic stresses (e.g., frost and salinity) and to decrease the impacts of biotic

stresses (e.g., pests, fungal diseases, and insect infestation) (Jayaraj et al. 2008, Rayorath et al. 2008). One of the most widely known seaweed-based biostimulants is Kelpak (Kelp Products Pty Ltd., Simon's Town, South Africa), which is a liquid biostimulant derived from the



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Effects of Microbubbles and Temperature on the Germination of the Saltmarsh Plant, *Suaeda Malacosperma*

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Abstract

Temperature and dissolved gas availability are important parameters for the germination of saltmarsh plants. As a newly developed method to increase the dissolved gas availability, the microbubble technology has shown to increase the crop germination and seedling growth. However, the effects of microbubbles (MBs) on the germination of saltmarsh plants have not been studied. The aim of this study is to investigate the effects of the MBs on the germination of. in different temperatures (6, 12, 18, 24 and 30°C). The highest germination percent was observed at 24°C in MBs water. The germination percent at MBs were significantly higher than those at control, deionized water at 24°C. The germination energy (GE) was significantly higher at 24 and 30°C than at 6 and 12°C in both deionized and MBs waters. The mean germination time (MGT) was significantly shorter at 24 and 30°C than at 6 and 12°C in both waters. However, no significant effects of MBs were observed in terms of GE and MGT. This study suggests that MBs improves the germination percent of *S. malacosperma* at the optimal temperature (24°C), but GE and MGT were not affected by MBs but by temperature in *S. malacosperma*.

Keywords Saltmarsh plants · *Suaeda malacosperma* · Seed germination · Temperature · Microbubbles

Introduction

Blue carbon is the carbon absorbed by the oceans and coastal ecosystems (Elizabeth et al. 2011; Siikamäki et al. 2013; Thorhaug et al. 2020). Globally, major blue carbon ecosystems include mangroves, seagrasses, and saltmarshes (Perillo et al. 2018; Kim et al. 2022b). Since mangroves don't exist in Korea, seagrass and saltmarsh ecosystems restoration projects have been conducted in this country. The saltmarshes in Korea occupy about 2.7% of the total west and south coasts (Sondak and Chung 2015). Therefore, using the huge saltmarsh area, the Ministry of Oceans and

Fisheries (MOF) of Korea has a goal to absorb and store more than 1 million tons of greenhouse gas (GHG) by 2050 (Kim et al. 2022b). However, the saltmarsh habitats have been continuously decreased during the past decades due to coastal development (MOF, 2019). To overcome this issue, MOF is promoting a saltmarsh restoration project on an area of 660 km² of Korea (MOF, 2022). To assist this effort, there are many studies being conducted for the restoration and conservation of saltmarsh ecosystems in Korea (Lee et al. 2009, 2012; Koo et al. 2011).

Many environmental factors influence seed germination. Inter alia salinity and temperature are key factors regulating the germination of saltmarsh plants (Del Vecchio et al. 2018; Khan et al. 2000; Van Regteren et al. 2020). The optimum germination temperature is different in different species. For example, the optimal germination temperature for *Phragmites australis* is 10–25 °C (Greenwood and MacFarlane 2006), while *Suaeda maritima* germinates well at 20–25 °C (Cheong 2011). However, different populations of the same species also may show different optimal germination temperatures due to the acclimation to their original habitats (Gorai et al. 2006). Thus, it is critical to determine

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Population specific responses to temperature and nutrients in the bloom forming *Ulva prolifera*

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Abstract

Different populations of the same species may have different physiological responses to environmental factors due to the adaptation to their environment. We tested interactive effects of temperatures (10, 15, 20, 25, and 30 °C) and nutrients (low nutrients: 5 µM NO₃⁻ and 0.5 µM PO₄⁻ (LN); medium nutrients: 50 µM NO₃⁻ and 5 µM PO₄⁻ (MN); high nutrients: 500 µM NO₃⁻ and 50 µM PO₄⁻ (HN)) in three different *Ulva prolifera* strains (one Chinese and two Korean strains). The results showed that all three strains of *Ulva* survived within the temperature range of 10 to 30 °C. The photosynthetic rates of all strains increased with increasing temperature from 10 to 30 °C under MN. However, at the higher temperature (30 °C) there was a significant reduction in the photosynthetic rate under HN in all three strains. A positive relationships between tissue nitrogen (N) and chlorophyll or soluble protein were observed in all three strains. The Chinese strain showed the lowest C:N ratio but the highest photosynthetic rate and tissue N contents. Our results show that the bloom forming Chinese strain may have higher nutrient uptake and assimilation ability, leading to higher photosynthetic activity. The *Ulva* strains may have lower tolerance to higher temperature at high nutrients conditions. These results suggest that the physiological responses of *U. prolifera* to different temperature and nutrients conditions can be population-specific.

Keywords Nutrients · Population-specific · Temperature · *Ulva prolifera* · Chlorophyceae

Introduction

Ocean temperatures have increased over the past 4 decades and are expected to continuously increase. The regional, seasonal and diurnal changes of temperature are expected to increase by 3–7 °C by the end of the century (Stocker et al. 2014). The increase of temperature impacts marine life at all levels of biological communities, and also interacts with other environmental factors, such as salinity, nutrients,

light, etc. (Lüning 1990; Hurd et al. 2014; Bindoff et al. 2019; Samanta et al. 2019a). Higher temperatures pose a serious threat to marine macroalgal survival, growth and reproduction (Lüning 1990; Davison 1991; Martínez et al. 2012; Wernberg et al. 2016; Han et al. 2023; Xing et al. 2023). Increases in temperature will influence the activity of enzymes involved in nutrients assimilation and carbon fixation (Davison 1991; Berges et al. 2002; Hurd et al. 2014). Additionally, different populations of the same species may exhibit different temperature tolerance due to the adaptations to surrounding environments at their origins. For example, Chinese strains *Ulva* show a higher hypo-salinity tolerance (20 psu) than Korean strains under the same condition (Lüning 1990; Bao et al. 2022, 2023). Consequently, the responses of macroalgae to changing temperatures may be population-specific (Poloczanska et al. 2013; Ji et al. 2016; Wiens 2016).

Another major environmental issue in coastal areas is eutrophication (Lohman and Priscu 1992; Fei 2012; Latimer et al. 2014). Nitrogen (N), as the primary component of numerous molecules, is integral to most biological processes such as the enzyme RUBISCO with carbon assimilation

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Regenerative aquatic foods can be a win-win for human and planetary health



The urgent global challenges of malnutrition, climate change and environmental degradation necessitate creating new, equitable, innovative and sustainable food system pathways¹. Regenerative aquatic foods (RAFTs), encompassing seaweeds, bivalves and pelagic small fish, represent a promising avenue, offering nutrient-rich foods while supporting ecological health. Compared with terrestrial foods, RAFTs often have equal or higher protein and nutritional value with considerably lower greenhouse gas emissions and reduced land and freshwater usage. Seaweeds, bivalves and pelagic small fish, in particular, present a unique opportunity to advance at least 7 of the 17 Sustainable Development Goals (SDGs). These foods are pivotal for sustainable food security in a climate-impacted world, providing essential nutritional benefits with minimal environmental footprints and are crucial contributors to global food equity^{2,3}.

During a convening at the Bellagio Center in July 2023, a group of experts co-created the RAF Roadmap, aligning closely with the SDGs. The experts, with backgrounds in nutrition, environmental conservation, aquaculture, policy development economics and community practices, recognized the need for a universal cross-organizational effort to advance regenerative aquatic foods, emphasizing sustainability and equitable food systems. It was agreed that RAFTs are aquatic foods from systems that enhance ecosystems, rather than deplete, and also offer restorative benefits, fostering positive relationships between people and nature^{4,5}. A four-dimensional (4D) framework of sustainable food systems (Fig. 1) was employed to identify the diverse benefits of RAFT across nutrition, planetary, sociocultural and economic dimensions.

This roadmap⁶ identifies strategic areas essential for upscaling RAF production sustainably:

- Consumption: integrate RAFTs into diets by creating appealing products, gaining endorsements from food champions, including RAFTs in dietary guidelines and



Fig. 1 | Four dimensions of sustainable food systems. Reproduced with permission from Food and Planet's 4D Framework, www.foodandplanet.org.

food composition tables, and supporting efforts in consumer education⁷.

- Community-based practices: enable local and Indigenous communities to steward and benefit from RAF resources, enhancing food sovereignty and sustainable management^{2,7}.
- Ecosystem services: monetize services such as carbon cycling, species recovery and nutrient reduction to promote economic systems that value ecological restoration and conservation^{8,9}.
- Capital investment: encourage innovative, environmentally friendly production methods through sufficient funding and effective financial mechanisms².
- Technology and innovation: utilize advanced technologies (for example, environmental monitoring and forecasting technology, and native species breeding programs) to optimize production and participatory incentive structures designed by local communities to ensure sustainability.

The RAF Roadmap aims to catalyse collective, equitable action through investments in consumer demand, community

empowerment, recognition of ecosystem services and technological innovation. RAF provides an actionable pathway that complements existing sustainability initiatives, promoting greater progress in restoring public health and the planet's resources. To make this roadmap a success, participation from diverse stakeholders is essential, including policymakers, researchers, community leaders, Indigenous knowledge holders, funders and private sector actors.

This Correspondence seeks to stimulate further dialogue and initiatives in the readership, encouraging diverse stakeholders to contribute to a sustainable and nutritious future. We must learn from past mistakes, such as the proliferation of monocultures and the under-valuation of Indigenous knowledge, to ensure a resilient and sustainable future. Implementing the RAF Roadmap is urgent – particularly for climate change mitigation and achieving the 2030 SDGs.

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ORIGINAL RESEARCH



WILEY

Effects of temperature and light on early sporophyte development of *Saccharina japonica* and *S. latissima* (Phaeophyta)

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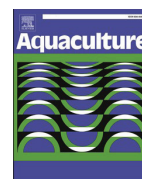
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Abstract

Saccharina japonica is common aquaculture species in Asia, whereas *S. latissima* is cultivated in North America and Europe. This study aims to select superior strains using breeding techniques for high-temperature tolerance and faster development at the early sporophytic stage. Qualitative experiments were conducted to observe the reproduction and early development of sporophytes under different temperatures (5, 10, 15, and 20°C) and light conditions (5 and 40-μmol photons m⁻² s⁻¹) for 20 days after male and female gametophytes were crossed. Two female (F05 and F15, *S. japonica*; FB and FO, *S. latissima*) and male (M06 and M14, *S. japonica*; MB and MO, *S. latissima*) gametophyte strains in each species were used. No inter-specific crosses were made. Four possible intraspecific crosses were cultured. Regardless of the species, the development of sporophytes was observed earlier at 10°C than all other

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Salinity alternation with dark treatment enhances the gel quality and quantity of carrageenan in a red alga, *Chondrus crispus*

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ABSTRACT

Alkali treatment is a commonly used method for carrageenan extraction in the industry. However, this method has some disadvantages, including reduced carrageenan yield and the generation of effluent. To address these issues, this study aimed to determine if salinity alternation combined with dark treatment could improve the yield and quality of carrageenan in the red alga, *Chondrus crispus* and whether this approach could serve as an eco-friendly alternative to alkali treatment. The results demonstrated that salinity alternation, coupled with dark treatment, enhanced both carrageenan yield and gel strength. Specifically, the most effective method was found to be salinity alternation from 70 psu for 10 days to 10 psu for an additional 10 days, combined with a 20-day of dark treatment period. These findings suggest that salinity alternation along with dark treatment could be a viable alternative to alkali treatment for *C. crispus*. However, further large-scale studies are needed to assess the economic feasibility of this eco-friendly method.

1. Introduction

The biosynthesis of carrageenans occurs in the order of Gigartinales, Rhodophyta. Some species within Gigartinales, including *Chondrus crispus* produce different types of carrageenan depending on their life cycle. The gelling kappa (κ) - and iota (ι)-carrageenan are synthesized by gametophytes, while the non-gelling λ-carrageenan is produced by sporophytes. Additionally, a hybrid form κ- and ι-carrageenan occurs in nature (Bixler et al., 2001; Falshaw et al., 2001; Villanueva et al., 2004). This κ/ι-hybrid carrageenan has received significant attention due to its high economic value and its gelling properties, which are beneficial for both food and non-food applications (de Vries, 2002; Falshaw et al., 2001; Falshaw et al., 2003; van de Velde, 2008; Villanueva et al., 2004).

Carrageenan consists of a disaccharide backbone with repeating α-1,3 and β-1,4-linked D-galactopyranose units (D and G units, respectively; Fig. 1a) (Anderson et al., 1968; Craigie, 1990; Guibet et al., 2007; Rees, 1965). κ- and ι-carrageenans are synthesized by substituting 3,6-anhydro-D-galactose (DA) units for the D units of the precursor, a process facilitated by sulfohydrolase or sulfotransferase enzymes in vivo (Fig. 1b, c). These carrageenans are classified based on the position and number of sulfate groups (Lawson and Rees, 1970; Rees, 1961, 1972; Wong and Craigie, 1978). Consequently, κ- carrageenan is represented

as the letter code G4S-DA, while ι-carrageenan is represented as G4S-DA2S. Environmental factors and extraction methods can affect the quantity and quality of carrageenan (Ali et al., 2018; Azevedo et al., 2013; Chopin et al., 1995; Knutsen et al., 1995; McCandless and Craigie, 1974; Simpson and Shacklock, 1979).

Chondrus crispus Stackhouse (Irish moss; Gigartinales, Rhodophyta) is a carrageenophyte (Santos and Duarte, 1991) and occurs in the Atlantic Ocean, primarily around Europe and North America. Its habitats have also been reported in South America (Argentina), Africa (Angola, Ghana, Libya, Mauritania), and the Pacific Ocean (Alaska, Bering Sea, Russia, Japan, Korea and Taiwan) (Guiry and Guiry, 2024). *C. crispus* is found throughout intertidal rocky shores where seaweeds experience salinity stress (high or low) due to evaporation and/or the inflow of freshwater from rain (Goulard et al., 2001; Hu et al., 2010; Provan et al., 2013). Floridoside and floridean starch are known to interfere with the synthesis of polysaccharides such as carrageenan and agar in red seaweeds. Under hypersaline conditions, the concentration of floridoside increases because floridean starch is converted into floridoside as an organic osmolyte (Dickson and Kirst, 1987; MacIer, 1988; Reed, 1985; Hayashi et al., 2011; Kirst, 1990; MacIer, 1986; REED, 1990). Consequently, when the salinity decreases to a hyposaline condition, floridean starch and floridoside levels decreases due to increase

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Physiological responses of *Grateloupia turuturu* and *Chondrus ocellatus* to nano-plastics

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ABSTRACT

The objective of this study is to investigate the effect of nano-plastics (NPs) on the growth, photosynthesis, oxidative stress and antioxidant enzymes in *Grateloupia turuturu* and *Chondrus ocellatus*. Difference of surface characteristics between *G. turuturu* and *C. ocellatus* may affect adherence of plastics to their surface. The seaweed samples were cultivated at 5 different NP concentrations (0, 20, 200, 2000, 20000 ng/L) for 21 days. The accumulation of nano-plastics on surface of *C. ocellatus* was higher than that of *G. turuturu*. The highest concentration of NPs (20000 ng/L) inhibited the growth and photosynthesis activity of *C. ocellatus*. At the same concentrations, oxidative stress was caused with increase of antioxidant enzyme activities. *G. turuturu* was not affected by NPs at all tested concentrations. Based on these results, toxic effects of nano-plastics may be species specific. Toxicity is dependent on the capacity of macroalgae to accumulate nano-plastics on their surface.

1. Introduction

Plastics have been manufactured and used globally for over a half-century (Fadare et al., 2019; Khoshnamvand et al., 2021). Global production of plastics has increased significantly over the past 70 years (e.g. 1.5 million metric tons (Mt) in the 1950s to 391 million Mt in 2021) (Auta et al., 2017; Okoffo et al., 2021; Statista, 2021; Tiseo, 2022). While the proportion of recycled plastics was less than 10% (Geyer et al., 2017; Maes et al., 2021), most discarded plastics can be transported through waterways and finally reach the ocean (Ter Halle et al., 2017). Plastic debris accounts for nearly 85 % of marine litter and can be accumulated for a long period because most plastics are persistent and non-biodegradable (Thompson et al., 2009). Plastic litter can affect the tourism industry, and entanglement of plastics can damage equipment for shipping, fishing, energy production and aquaculture (Derraik, 2002; Barnes et al., 2009; Sivan, 2011). Marine birds, mammals, fish and reptiles can also be injured and killed by ingestion and entanglement of plastics (Gregory, 2009; Lopez Lozano and Mouat, 2009). Additionally, floated plastic litter can be a vector for invasive species (Barnes, 2002). Thus, plastic litter has been considered as a serious pollutant in the global oceans (Maes et al., 2021).

Plastics are degraded into micro- or nano-plastics in natural

environment by photodegradation, thermal oxidation degradation, hydrolysis, and physical degradation (Mattsson et al., 2021; Sui et al., 2022). Nano-plastics are defined as particle size <100 nm or <1000 nm in different studies (Wang et al., 2020b). Due to technological limitations and dilution effects, few studies have reported the actual concentrations of nano-plastics in the ocean and interactions between nano-plastics and marine organisms (Ter Halle et al., 2017). Nano-plastics have higher surface area to volume (SA:V) ratio than micro-plastics. Higher SA:V ratio induces the abilities to interact with organisms and therefore causes negative effects (Jeong et al., 2016). Thus, nano-plastics may have higher toxicity to marine organisms than micro-plastics (Nolte et al., 2017; Chae et al., 2018). Nano-plastics also have a potential to be transported into cells due to their small size (Rossi et al., 2014; Forte et al., 2016). Many studies have reported that micro- and nano-plastics can be ingested by various trophic levels of marine animals (Derraik, 2002). However, little is known about the interactions between the plastics and photosynthetic organisms, especially macroalgae. Nano-plastics can attach to the surface of algae and be transported through the food chain. In some cases, adherence of nano-plastics on microalgae caused growth inhibition and decrease of photosynthetic activity with reactive oxygen species (ROS) production (Bhattacharya et al., 2010; Besseling et al., 2014; Sjollem et al., 2016; Bergami et al.,

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Research Article

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Effects of environmental and physical factors on the shell infiltration density of free-living conchocelis of three *Pyropia* species (Bangiales, Rhodophyta)

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Shell infiltration of free-living conchocelis of the three commercial *Pyropia* species (*P. yezoensis*, *P. seriata*, and *P. dentata*) were examined in various conditions of temperatures (20–25°C), light intensities (1, 10, 40, and 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), conchocelis quantities (100, 150, 200, 250, and 300 mg) and sizes (108, 209, 324, 411, and 519 μm). The optimal shell infiltration conditions were 20°C and 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in all species. The infiltration densities were 44.81–146.15 conchocelis cm^{-2} for *P. yezoensis*, 51.40–190.49 conchocelis cm^{-2} for *P. seriata*, and 22.24–92.49 conchocelis cm^{-2} for *P. dentata*. Inefficient shell infiltration was observed with the different conchocelis quantities from 100 to 300 mg. A positive correlation was observed between shell infiltration density and the amount of conchocelis. The optimal blending time for maximizing infiltration density was 40 s for *P. yezoensis* and *P. seriata* and 25 s for *P. dentata*. This blending time correlated with a cell count of 5–7 cells. Total conchocelis lengths of 5–7 cells were approximately 300 μm for *P. yezoensis* and *P. seriata* (with cell size of about 45 μm) and approximately 400 μm for *P. dentata* (with a cell size of about 65 μm). These results suggest that, to achieve optimal shell infiltration density for *P. dentata*, the blending time should be reduced by half, and the amount of conchocelis should be increased twofold, making it comparable to the other two *Pyropia* species.

Keywords: free-living conchocelis; *Pyropia dentata*; *Pyropia seriata*; *Pyropia yezoensis*; shell infiltration density

INTRODUCTION

Pyropia is an economically important and widely cultivated seaweed in Asia, such as Korea, Japan, and China (He et al. 2021, Kim et al. 2022, Zuccarello et al. 2022, Zhong et al. 2023). The main cultivated *Pyropia* species are *P. tenera*, *P. yezoensis*, *P. seriata*, *P. dentata*, and *P. haitanensis* (Hwang et al. 2005, Luo et al. 2014, He et al. 2023, Kim et al. 2023). In Korea, four *Pyropia* species except *P. haitanensis*, are cultivated, but *P. tenera* is no longer farmed due to its low productivity and fertility (Hwang et

al. 2010). As commercial production continues to grow, *Pyropia* is increasingly used not only as food but also in medicine and the chemical industry (Harada et al. 1997, Choi et al. 2016, Oh et al. 2018, Park et al. 2023, Wang et al. 2023).

In Korea, the *Pyropia* aquaculture industry has maintained its production level of 507,362 to 575,634 tons (wet weight) and economic value of US\$332–400 million over the past three years (2021–2023). For the 2024 period



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Effects of microplastics and phenanthrene on gut microbiome and metabolome alterations in the marine medaka *Oryzias melastigma*

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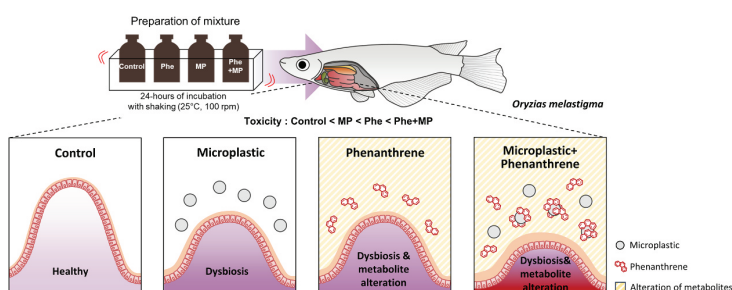
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HIGHLIGHTS

- Co-exposure of phenanthrene (Phe) and microplastic (MP) induced synergistic toxicity.
- Exposure to Phe and/or MP led to dysbiosis of the gut microbiota.
- Synergistic effects of Phe and MP were revealed by microbiome and metabolome analysis.
- Correlations between microbiota and metabolites were identified upon combined exposure.

GRAPHICAL ABSTRACT



ARTICLE INFO

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Microplastics
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Microbiome
Metabolome

ABSTRACT

Plastic pollution of the oceans is increasing, and toxic interactions between microplastics (MPs) and organic pollutants have become a major environmental concern. However, the combined effects of organic pollutants and MPs on microbiomes and metabolomes have not been studied extensively. In the present study, to evaluate whether MPs and phenanthrene (Phe) act synergistically in the guts of marine medaka (*Oryzias melastigma*), we performed toxicity assessments, 16 S rRNA gene sequencing, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. Our investigations revealed increased toxicity induced by Phe, as well as disturbances in gut microbiota (known as dysbiosis) when MPs were present. Furthermore, combined exposure to Phe and MPs resulted in greater alterations to microbiota composition and metabolite profiles. Notably, MP exposure was distinctly associated with the abundance of *Shewanella* and *Sphingobacteriaceae*, while Phe exposure was associated with the abundance of *Marimicrobium*. Among key microbiota, *Marimicrobium* and *Roseibacillus* were significantly correlated with metabolites responsible for coenzyme A and glycerophospholipid metabolism in medaka. These results suggest that interactions between Phe and MPs may have significant effects on the gut microbiota and metabolism of aquatic organisms and underscore the importance of acknowledging the interplay between MPs and contaminants in aquatic environments.

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Review

Environmental occurrence, biological effects, and health implications of zinc pyrithione: A review

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ARTICLE INFO

Keywords:

Antifoulant

Environmental fate

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ABSTRACT

Due to the detrimental effects on aquatic organisms and ecosystem, tributyltin as an antifouling agent have been banned worldwide since 1990s. As a replacement for tributyltin, zinc pyrithione (ZnPT) has emerged as a new environmentally friendly antifouling agent. However, the widespread use of ZnPT unavoidably leads to the occurrence and accumulation in aquatic environments, especially in waters with limited sunlight. Despite empirical evidence demonstrating the ecotoxicity and health risks of ZnPT to different organisms, there has been no attempt to compile and interpret this data. The present review revealed that over the past 50 years, numerous studies have documented the toxicity of ZnPT in various organisms, both *in vitro* and *in vivo*. However, long-term effects and underlying mechanisms of ZnPT on biota, particularly at environmentally realistic exposure levels, remain largely unexplored. In-depth studies are thus necessary to generate detailed ecotoxicological information of ZnPT for environmental risk assessment and management.

1. Introduction

Marine biofouling organisms, which encompass bacteria, algae, and the floating larvae of invertebrates such as sea squirts, barnacles, and tubeworms, are widely present in marine environments and have long been a major challenge for the shipping industry. These fouling organisms adhere to and grow on the ship's hull (Lewis, 1998), leading to physical damage, reduced vessel speed, and increased fuel consumption (Abbott et al., 2000). To mitigate biofouling on ship surfaces, antifouling agents are therefore incorporated into protective paints. In the past, copper and tributyltin (TBT) were commonly used as antifouling agents, gradually leaching from the paint to inhibit the attachment and growth of marine biofouling organisms (Lewis, 1998). However, the extensive global use of TBT resulted in its presence at elevated environmental concentrations, causing detrimental effects on various marine organisms. Consequently, TBT was banned globally since the 1990s (Ebdon

et al., 1989; Antizar-Ladislao, 2008). Subsequently, alternative antifouling boosters such as chlorothalonil, diuron, dichlofluanid, Irgarol 1051, sea-nine 211, TCMS pyridine, TCMTB, and zineb, zinc pyrithione (ZnPT) have emerged in the last two decades (Konstantinou and Albanis, 2004; Dafforn et al., 2011; Guardiola et al., 2012).

Among the new type of antifouling agents, ZnPT is also an antibacterial drug approved by the United States Food and Drug Administration (FDA), widely used not only in clinical treatments but also added to various daily care products, including anti-dandruff shampoos, detergents, and cosmetics (Van Cutsem et al., 1990; Bailey et al., 2003). ZnPT can effectively inhibit the growth and proliferation of bacteria and fungal possibly through inhibition of protein synthesis and membrane transport (Chandler and Segel, 1978), depolarization of membrane gradient (Ermolayeva and Sanders, 1995), depletion of ATP (Dinning et al., 1998), induction of iron starvation (Yasokawa et al., 2010), accumulation of intracellular copper, disruption of iron-sulphur clusters

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Detection of the jellyfish *Chrysaora pacifica* by RPA-CRISPR-Cas12a environmental DNA (eDNA) assay and its evaluation through field validation and comparative eDNA analyses

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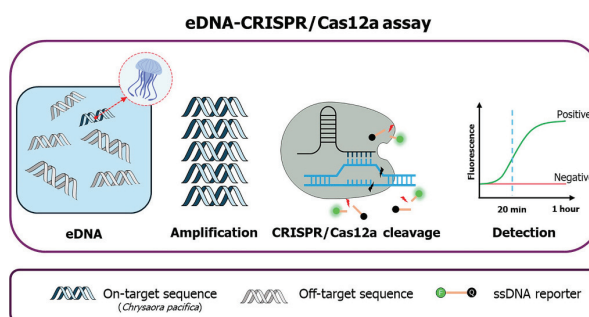
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HIGHLIGHTS

- RPA-CRISPR-Cas12a eDNA assay is highly sensitive and specific to target species.
- The assay was highly sensitive with two copies of *Chrysaora pacifica* COI/μL.
- Outcomes of assay were correlated with digital PCR in environmental DNA samples.
- The reliability of the assay was validated by traditional monitoring data of jellyfish.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Olga Pantos

Keywords:
eDNA
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Chrysaora pacifica
Jinhae Bay
dPCR
Metabarcoding

ABSTRACT

Climate-driven environmental changes and anthropogenic activities can result in the proliferation of non-indigenous aquatic species such as jellyfish that may cause envenomation and various ecological disruptions. Here we developed a two-step RPA-CRISPR-Cas12a eDNA assay, consisting of target eDNA amplification followed by a CRISPR-Cas12 reaction, for the early detection of *Chrysaora pacifica*, a jellyfish species often considered non-indigenous to South Korea. The assay demonstrated high sensitivity, with a detection limit of two copies COI/μL for eDNA derived from *C. pacifica*, using target specific RPA primers and crRNA sequences. Field validation of the assay using eDNA samples from Jinhae Bay collected over eight months of time-series monitoring, revealed temporal distribution of the jellyfish which correlated with results of digital polymerase chain reaction (dPCR) and eDNA metabarcoding. The *C. pacifica* eDNA assays were also corroborated (R-square 0.7891) by reports from a citizen science-based jellyfish-monitoring program operated by the National Institute of Fisheries Science, South Korea. Our RPA-CRISPR-Cas eDNA assay can therefore, be an efficient alternative to traditional tools for the early detection of outbreaks of non-indigenous or harmful species in marine ecosystems.

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Jeongyeon Heo and Hara Kang*

Platelet-derived growth factor-stimulated pulmonary artery smooth muscle cells regulate pulmonary artery endothelial cell dysfunction through extracellular vesicle miR-409-5p

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Abstract: Platelet-derived growth factor (PDGF)-induced changes in vascular smooth muscle cells (VSMCs) stimulate vascular remodeling, resulting in vascular diseases such as pulmonary arterial hypertension. VSMCs communicate with endothelial cells through extracellular vesicles (EVs) carrying cargos, including microRNAs. To understand the molecular mechanisms through which PDGF-stimulated pulmonary artery smooth muscle cells (PASMCs) interact with pulmonary artery endothelial cells (PAECs) under pathological conditions, we investigated the crosstalk between PASMCs and PAECs via extracellular vesicle miR-409-5p under PDGF stimulation. miR-409-5p expression was upregulated in PASMCs upon PDGF signaling, and it was released into EVs. The elevated expression of miR-409-5p was transported to PAECs and led to their impaired function, including reduced NO release, which consequentially resulted in enhanced PASMC proliferation. We propose that the positive regulatory loop of PASMC-extracellular vesicle miR-409-5p-PAEC is a potential mechanism underlying the proliferation of PASMCs under PDGF stimulation. Therefore, miR-409-5p may be a novel therapeutic target for the treatment of vascular diseases, including pulmonary arterial hypertension.

Keywords: extracellular vesicle; microRNA; PDGF signaling; pulmonary artery endothelial cell; pulmonary artery smooth muscle cell

1 Introduction

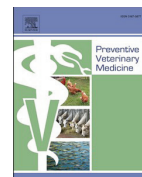
Platelet-derived growth factor (PDGF) is a mitogen that stimulates the proliferation and migration of vascular smooth muscle cells (VSMCs), which can induce the VSMC phenotype switching from a quiescent “contractile phenotype” to a proliferative “synthetic phenotype” (Tallquist and Kazlauskas 2004). Abnormal proliferation of VSMCs is a key feature in the development of vascular diseases, including atherosclerosis and pulmonary arterial hypertension (Barst 2005; Raines 2004; Schermuly et al. 2005). PDGF signaling can also cause inflammation of the aorta and architectural changes in the muscle layer of the aorta (He et al. 2015). Therefore, regulation of PDGF signaling is important for the maintenance of vascular homeostasis, and aberrant PDGF signaling is implicated in multiple vascular diseases.

Vascular pathologies include VSMC proliferation and migration, endothelial cell (EC) dysfunction, and inflammation (Barst 2005; Humbert et al. 2008; Libby et al. 2011). As the vessel wall consists of several cell types, including VSMCs, ECs, and fibroblasts, vascular pathogenesis progresses through intercellular communication (Su et al. 2017). Extracellular vesicles (EVs) are critical mediators involved in vascular cell communication (Loyer et al. 2014; Wang et al. 2022). By transferring a variety of cargos in EVs, including RNA, DNA, and proteins of donor cells, to recipient cells, EVs elicit functional effects on the recipient cells (Al-Nedawi et al. 2008; Raposo and Stoorvogel 2013; Valadi et al. 2007; Waldenstrom et al. 2012). The role of EVs in cardiovascular disease has been highlighted by recent investigations (Deng et al. 2015; Loyer et al. 2014; Su et al. 2017; Zheng et al. 2017). For instance, extracellular vesicle cargo exchange among VSMCs, ECs, and macrophages is known to regulate the progression of atherosclerosis (Chen et al. 2017; Heo and Kang 2022; Li et al. 2016, 2020).

To understand intercellular communication using EVs that are associated with vascular diseases, it is important to explore differential cargos in EVs derived from vascular cells under normal and PDGF-stimulated conditions because microenvironmental stimuli can influence the contents of EVs. As miRNAs mediate cellular communication in the pulmonary vascular wall, we compared the expression

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Risk factors associated with *Coxiella burnetii* in wild boars: A study in South Korea

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Keywords:

Coxiella burnetii
Infectious disease
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Risk factor
Wild animal

ABSTRACT

Coxiella burnetii is a Gram-negative bacterium that causes the zoonotic disease Q fever. Wild boars serve as reservoirs for *C. burnetii*. This study aimed to identify the risk factors associated with *C. burnetii* infection in wild boars. We analyzed the data from 975 wild boar samples collected from June to November 2021 in South Korea. We utilized the indirect ELISA to detect antibodies against *C. burnetii*. A sample optical density to positive-control optical density value exceeding 50% was classified as positive. We gathered data on the forestation, terrain, weather, agriculture, and animal density of the region where the samples were collected. Continuous variables were categorized into tertiles. We performed a univariate logistic regression analysis and included variables with a p-value < 0.2 in the final multivariable logistic regression model. In our multivariable logistic regression analysis to identify risk factors for *C. burnetii* infection in wild boars, we used a forward selection method to enter variables based on the order of their significance. We performed the final multivariable logistic regression analyses using either continuous variables or variables categorized into tertiles. The prevalence of *C. burnetii* was 14.6% (n=142). Locations with the highest maximum wind speeds (3.92–8.24 m/s) showed a 59% increase in infection odds compared to locations with the lowest speeds (1.45–3.25 m/s) (p=0.044). For each 1 m/s increase in maximum wind speed, infection odds increased by 24.1% (p=0.037). Regions with the highest percentage of paddy fields per area (8.3–45%) showed a 76% increase in infection odds compared to regions with the lowest percentage (0–1.5%) (p=0.011). For each 1% increase in the proportion of paddy fields per area, infection odds increased by 3.3% (p=0.003). High maximum wind speed and a high percentage of paddy field were identified as significant risk factors for *C. burnetii* infection in wild boars.

1. Introduction

Coxiella burnetii is a Gram-negative bacterium that causes Q fever, a zoonotic disease that is usually asymptomatic or presents with mild flu-like symptoms (Derrick, 1983; Maurin and Raoult, 1999). However, in pregnant female animals, the bacterium can cause reproductive problems such as abortion and low birth weight (Roest et al., 2012; Álvarez-Alonso et al., 2018).

Various mammals and birds serve as primary reservoirs of *C. burnetii*, including both domestic species and wildlife (Celina and Cerny, 2022). The primary routes of *C. burnetii* infection are through ticks and

inhalation of contaminated aerosols or dust. Ticks transmit the pathogen by feeding on an infected host and subsequently passing it to the next host during later feedings (Maurin and Raoult, 1999). Transmission via aerosols or dust occurs when individuals inhale particles contaminated with placental materials, feces, or vaginal secretions from infected animals (Maurin and Raoult, 1999). The mode of transmission through aerosols or dust can potentially disperse over long distances, covering approximately 18 km (Hawker et al., 1998; Tissot-Dupont et al., 2004). In fact, humans within a 5 km radius of infected livestock farms are at risk of infection (van der Hoek et al., 2011b; Clark and Soares Magalhães, 2018).

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Coding-complete genome sequence and phylogenetic analysis of Coxsackievirus B3 isolated from South Korea

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AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Whole-genome sequencing of a Coxsackievirus B3 strain isolated from the stool of a febrile patient with aseptic meningoencephalitis, South Korea, in 2002 was performed. This strain exhibits a high nucleotide sequence identity with various strains circulating in China from 2001 to 2019.

KEYWORDS Coxsackievirus B3, genome, *human enterovirus*, phylogenetic analysis

Coxsackievirus B3 (CVB3), a member of the *human enterovirus B* species, belongs to the genus *Enterovirus* in the family *Picornaviridae* (1). CVB3 is mainly associated with aseptic meningitis and pericarditis; there have been reported cases of global transmission causing pancreatic and gastrointestinal disorders (2–6), acute flaccid paralysis (7), and more recently, hand, foot, and mouth disease (8–10). Despite its significance, to our knowledge, no genetic analysis of the whole-genome sequence of CVB3 strains isolated in South Korea has been reported (Table 1). Thus, this study aims to bridge this gap by conducting a thorough genomic analysis, determining the coding-complete genome sequence of a CVB3 strain circulating in the country. Here, we present a coding-complete genome sequence of a CVB3 strain isolated in South Korea, enhancing our understanding of the genetic relatedness of CVB3 in the Asia-Pacific region.

CVB3 KR-2002 strain isolated from the stool of a febrile patient with aseptic meningoencephalitis in Jeollabuk-do, South Korea, in 2002 was acquired from the National Culture Collection for Pathogens (Osong-eup, Cheongju-si, South Korea). The virus was serially passed five times in Vero cells, and the virus was harvested from the infected cell cultures before RNA extraction using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA). Sequencing libraries were prepared using the Illumina Stranded Total RNA prep ligation with Ribo Zero Plus Library prep kit (Illumina, San Diego, CA, USA) and sequenced on an Illumina Miseq 300 bp paired-end reads. The quality control of the 8,576,486 reads was assessed using FastQC v0.11.9, and the adapters were trimmed using Trimmomatic v0.39 with the following parameters: ILLUMINACLIP:2:30:10:2:keep-BothReads LEADING:10 TRAILING:10 MINLEN:100 (11, 12). The CVB3 whole genomes were then *de novo* assembled using SPAdes 3.15.2 (13). All tools were run with default parameters unless otherwise specified.

The coding-complete genome of the CVB3 KR-2002 strain was found to contain 7,386 nucleotides (nt), excluding the poly (A) tail. The 5′ untranslated region (UTR) was found to be 731 nt, followed by a single open reading frame (ORF) encoding a large polyprotein (2,186 amino acids), and the 3′ UTR was 97 nt long. The CVB3 KR-2002 strain genome consists of 28.12% A, 22.98% C, 24.51% G, and 24.4% U with a G + C content of 47.49%. Phylogenetic analysis using the maximum likelihood method based on the coding-complete genome reveals that KR-2002 CVB3 strain belongs to the genogroup D (Fig. 1) (10). The CVB3 KR-2002 strain is more closely related to CVB3 2001-5 strain from China of genogroup D (GenBank accession no. [MK791148.1](#); 91% nucleotide identity in ORF). As shown in Fig. 1, genogroup D consists almost exclusively of strains isolated in

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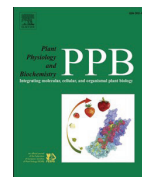
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Integrated “-omics” analysis highlights the role of brassinosteroid signaling and antioxidant machinery underlying improved rice seed longevity during artificial aging treatment

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Brassinosteroids
Heat shock proteins

ABSTRACT

Seed longevity is a critical characteristic in agriculture, yet the specific genes/proteins responsible for this trait and the molecular mechanisms underlying reduced longevity during seed aging remain largely elusive. Here we report the comparative proteome and metabolome profiling of three rice cultivars exhibiting varying degrees of aging tolerance: Dharial, an aging-tolerant cultivar; Ilmi, an aging-sensitive cultivar; and A2, a moderately aging-tolerant cultivar developed from the crossbreeding of Dharial and Ilmi. Artificial aging treatment (AAT) markedly reduced the germination percentage and enhanced the activities of antioxidant enzymes in all the cultivars. Further, proteomics results showed a key role of the ubiquitin (Ub)-proteasome pathway in the degradation of damaged proteins during AAT while other proteases were majorly reduced. In addition, proteins associated with energy production and protein synthesis were strongly reduced in Ilmi while these were majorly increased in A2 and Dharial. These, along with metabolomics results, suggest that Ub-proteasome mediated protein degradation during AAT results in the accumulation of free amino acids in Ilmi while tolerant cultivars potentially utilize those for energy production and synthesis of stress-related proteins, especially hsp20/alpha-crystallin family protein. Additionally, both Dharial and A2 seem to activate brassinosteroid signaling and suppress jasmonate signaling which initiates a signaling cascade that allows accumulation of enzymatic and non-enzymatic antioxidants for efficient detoxification of aging-induced ROS. Taken together, these results provide an in-depth understanding of the aging-induced changes in rice seeds and highlight key pathways responsible for maintaining seed longevity during AAT.

1. Introduction

The ability of a seed to maintain its viability during dry storage, known as seed longevity, is a crucial trait in preserving plant diversity and genetic resources in agriculture (Zhang et al., 2021). Different crops exhibit varying levels of seed longevity and tolerance to aging, with some losing viability quickly under natural aging while others remaining viable for several years. External factors such as temperature, humidity, and other environmental conditions during seed maturation, harvesting, and storage can facilitate the process of seed aging.

Therefore, efforts have been made in the past to understand the process of seed aging by exposing the matured seeds to artificial aging treatment (AAT), also known as control deterioration treatment (CDT) or accelerated aging treatment, which refers to the treatments given to the seeds to promote aging. AAT is a valuable tool and thus has been frequently utilized to investigate molecular changes during seed aging. High temperature and high humidity conditions during AAT promote the accumulation of reactive oxygen species (ROS) which causes cellular oxidation and promotes denaturation and aggregation of seed proteins. Notably, ROS-induced enzyme deactivation results in attenuation of

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Article

Metabolomics Reveals *Lysinibacillus capsici* TT41-Induced Metabolic Shifts Enhancing Drought Stress Tolerance in Kimchi Cabbage (*Brassica rapa* L. subsp. *pekinensis*)

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Abstract: Climate change has increased variable weather patterns that affect plants. To address these issues, we developed a microbial biocontrol agent against drought stress in kimchi cabbage (*Brassica rapa* L. subsp. *pekinensis*). We selected three bacterial strains (*Leifsonia* sp. CS9, *Bacillus toyonensis* TSJ7, and *Lysinibacillus capsici* TT41) because they showed a survival rate of up to 50% and good growth rate when treated with 30% PEG 6000. The three strains were treated with kimchi cabbage to confirm their enhanced drought stress resistance under non-watering conditions. Among the three strains, the TT41 treated group showed a significant increase in various plant parameters compared with the negative control on the 7th day. We performed extensive profiling of primary and secondary metabolites from kimchi cabbage and the TT41 strain. Multivariate and pathway analyses revealed that only the TT41 group clustered with the well-watered group and showed almost the same metabolome on the 7th day. When treated with TT41, lactic acid was identified as an indicator metabolite that significantly improved drought stress tolerance. Furthermore, lactic acid treatment effectively induced drought stress tolerance in kimchi cabbage, similar to that achieved with the TT41 strain.

Keywords: drought stress; abiotic stress; lactic acid; metabolomics; *Brassica rapa* L.; cabbage



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1. Introduction

Weather patterns associated with climate change, such as droughts, floods, and extreme temperatures, have increased over the past decades [1]. These climate events are expected to cause significant crop losses. This situation poses a threat to global food security and the growing food demand. Therefore, strategies to improve food tolerance in abiotic environments are essential. Various innovative breeding and biotechnological approaches have been developed to improve plant abiotic stress tolerance.

Plant root-associated microorganisms can positively influence plant tolerance to abiotic stresses [2]. It is well-established that diverse bacteria and mycorrhizal fungi can augment stress tolerance in numerous plants [3]. Various species of bacteria and fungi have been reported to promote the growth and productivity of plants and to control plant pathogens under laboratory conditions [4]. Plant growth-promoting *Rhizobacteria* (PGPRs) are soil bacteria that directly or indirectly promote plant rooting and growth of plants [5]. PGPRs influence plant growth through the fixation of atmospheric nitrogen, solubilization of insoluble phosphates, secretion of phytohormones such as indole-3-acetic acid (IAA) and gibberellin (GAs), kinetics, and production of exopolysaccharides. However, when PGPRs are used in fields exposed to natural environmental conditions, they are highly



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Influence of medium and gelling agents concentration on *in vitro* rooting of *Polygonum tinctorium*

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ABSTRACT

Polygonum tinctorium has long been employed in the medical and dye industries in many nations, particularly in Korea and Japan, for traditional fabric staining as a source of blue color. The plant tissue culture method has proven to be a rapid and sustainable approach for the regeneration of various plant species, particularly those with medicinal or ornamental value. In this study, we explored the *in vitro* root regeneration and growth of *P. tinctorium* in response to different growth media and gelling agents. Among the tested media, Schenk and Hildebrandt medium (SH medium) demonstrated superior performance, resulting in the highest number and length of roots per explant. SH media produced about 53 and 18% higher root per explant and a 40% and 14% increase in root length compared to B5 and MS media, respectively. Half strength of SH medium proved to be the optimal condition for both root number (7.46 roots per explant) and root length (3.81 mm). Among the gelling agents, 2 g/L of Gelrite medium was most effective in promoting the highest and tallest roots. These findings have the potential to enhance rooting abilities in various crops, particularly in the case of medicinal and ornamental plants, and may offer valuable insights for future industrial-scale root production of *P. tinctorium*.

KEYWORDS: Gelling agents, Root regeneration, Growth media, *In vitro* rooting ability

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INTRODUCTION

The annual plant *P. tinctorium* has long been employed in the medical and dye industries (Cooksey, 2007; Hirota *et al.*, 2014). It is also often used in many nations, particularly Korea and Japan, for traditional fabric staining as a source of blue color (Chung *et al.*, 2005; Hirota *et al.*, 2016). A substance called indigo, which was discovered from *P. tinctorium*, is widely used in traditional Chinese medicine and as a natural color in denim manufacturing (Kukuła-Koch *et al.*, 2013). The greatest quantities of indigo for therapeutic purposes have been extracted from naturally existing plant material using water extraction and alkaline precipitation (Campeol *et al.*, 2006).

Plant tissue culture methods are now widely employed as a valuable tool for fundamental and applied research as well as for the large-scale production of commercially significant crops (Shasmita *et al.*, 2017; Ozdemir & Budak, 2018). All plants,

whether they may be trees, shrubs, or herbs, are often propagated from their seeds. Plants can be multiplied by different vegetative methods in addition to seeds. However, seed regeneration requires a long period and is regarded as a low-propagation mode of regeneration. On the other hand, tissue culture methods are the exact reverse of conventional regeneration methods in that they may be quickly and massively multiplied for clonal regeneration, genetic enhancement, and the conservation of endangered species (Park *et al.*, 2009).

The fundamental concept in a micropropagation system revolves around acclimation, with the final stage of the culture process referred to as rooting (Ismail *et al.*, 2011; Millán-Orozco *et al.*, 2011). In order to thrive in natural settings, *in vitro*-generated plantlets need a robust root system that contributes to the soil's absorption of water and nutrients (Benková & Bielach, 2010). Application of exogenous natural or synthetic auxins may facilitate the rooting process (Osterc & Štampar, 2011),

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Article

Effects of Different Solvents on the Extraction of Phenolic and Flavonoid Compounds, and Antioxidant Activities, in *Scutellaria baicalensis* Hairy RootsJinsu Lim ^{1,2} , Kihyun Kim ³, Do Yeon Kwon ², Jae Kwang Kim ⁴ , Ramaraj Sathasivam ^{3,*} and Sang Un Park ^{1,3,5,*}

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Abstract: The hairy roots (HRs) of *Scutellaria baicalensis* are widely used and consumed worldwide as a medicine, especially in Asian countries, due to their biological and pharmacological activities. The HRs of this plant are rich in secondary metabolites. However, the optimal method and solvents for the extraction of secondary metabolites from *S. baicalensis* HRs have not been well studied. Therefore, in this study, *S. baicalensis* HRs were extracted with different solvents, including water (WE), 99.9% pure methanol (PM), 70% aqueous methanol (AM), 99.9% pure ethanol (PE), and 70% aqueous ethanol (AE). The phenolic and flavonoid compounds and various antioxidant activities of each extract were measured. The AE extract (16.85 ± 0.15%) had a higher yield, which led to the highest accumulation of total phenolic content (TPC) and total flavonoid content (TFC), and antioxidant activity. The TPC and TFC were highest in AE (66.03 ± 0.44 mg GAE/g and 40.11 ± 1.31 mg QE/g, respectively), whereas WE, PM, and PE showed lower values in all assays. In addition, the highest antioxidant activities, such as DPPH, ABTS, and SOD-like scavenging activities and reducing power, were achieved in the AE extract compared to the other solvent extracts. Based on these results, the AE extract showed the highest phenolic and flavonoid accumulation and antioxidant activities, highlighting its potential use in the manufacture of useful materials from *S. baicalensis* HRs and its novel applications.

Keywords: *Scutellaria baicalensis*; extraction; solvents; phenolics; flavonoid; antioxidant activities



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1. Introduction

In recent years, an increasing number of scientists have reached the consensus that oxidants play an important role in the ageing process, supported by compelling evidence [1]. Consequently, several synthetic antioxidants have been formulated and developed. However, these synthetic antioxidants cause harmful damage to vital organs, including the circulatory system, kidneys, liver, and stomach, leading to conditions such as cancer [2]. Therefore, this growing concern has led to an increase in interest in natural dietary alternatives to synthetic antioxidants, highlighting the current research into vegetable sources and the investigation of raw materials to improve novel antioxidants.

Scutellaria baicalensis Georgi, otherwise called Chinese skullcap, has an extensive history of therapeutic use across East Asia, such as China and Korea, as well as in Europe



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Molecular characterization of diosgenin biosynthesis in different organs of *Trigonella foenum-graecum* L.

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ABSTRACT

Fenugreek, an annual herb, is a significant source of diosgenin, a triterpene with bioactive properties. This study aimed to molecularly characterize diosgenin biosynthesis in various organs of fenugreek, including flowers, immature leaves, developing leaves, mature leaves, stems, roots, and pods. The biosynthetic pathway involves vital enzymes such as squalene synthase, squalene monooxygenase, cycloartenol synthase, and Sterol-3- β -glucosyl transferase. The study found that flowers and roots exhibited the highest gene expression levels, indicating their potential significance in diosgenin biosynthesis. Diosgenin content was quantified using high-performance liquid chromatography, with seeds being the primary source. The findings suggest that fenugreek offers a cost-effective and rapid alternative to yam for diosgenin production, and the differential accumulation of diosgenin in different plant organs underscores the importance of understanding organ-specific biosynthesis. This comprehensive analysis provides valuable insights into fenugreek's potential as a bioresource for diosgenin and its diverse applications in medicine and agriculture.

KEYWORDS: *Trigonella foenum*, Bioactive properties, Mature leaves, Diosgenin, Potential, Biosynthesis, Squalene

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INTRODUCTION

The annual herb fenugreek, or *Trigonella foenum-graecum* L. (Fabaceae), is widely grown from Iran to Northern India and also in Egypt, Southern Europe, China, and other places (Snehlata & Payal, 2012). Fenugreek leaves and seeds are significant reservoirs of steroidal sapogenins, particularly diosgenin, used in medicine. These sapogenins have been widely produced in extracts and powders (Acharya *et al.*, 2006; Mehrafarin *et al.*, 2010). Moreover, they show various beneficial effects, such as their antidiabetic effects and their nutritional and therapeutic properties (Mozaffari *et al.*, 2010). Fenugreek comprises secondary metabolites, including steroidal saponins, flavonoids, phenolic compounds, and alkaloids, among other chemicals (Laila & Murtaza, 2015; Wani & Kumar, 2016). The important bioactive chemical molecule diosgenin is found in fenugreek. It is a saponin with a structure similar to cholesterol and other steroids (Rahmati-Yamchi *et al.*, 2014).

Diosgenin is classified as a triterpene and is a significant bioactive compound. A Phyto steroid sapogenin that is naturally made and can be found in yams, fenugreek, and *Costus speciosus* (Liu *et al.*, 2012; Kumar *et al.*, 2014). Terpenes are made from the isoprene precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). This can be done through either the 2-C-methyl-d-erythritol 4-phosphate (MEP) or the mevalonate (MVA) pathway. An enzyme called squalene synthase (SQS) helps two molecules of farnesyl pyrophosphate (FPP) make squalene, a C30 compound. Following this, a squalene epoxidase (SQE) helps this compound undergo epoxidation, which creates 2,3-oxidosqualene. The cycloartenol synthase (CAS) enzyme catalyzes the conversion of 2,3-oxidosqualene into cycloartenol. Cycloartenol is the initial compound for synthesizing many plant steroids, such as diosgenin (Thimmappa *et al.*, 2014).

Yam is an essential source of diosgenin, but its production is expensive and time-consuming. In contrast, fenugreek has a

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A Comprehensive Metabolite Profile of Green and White Asparagus (*Asparagus officinalis* L.) and Their Antioxidant Properties in Differing Cultivation Conditions

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Abstract

Asparagus (*Asparagus officinalis* L.) is a food crop with a variety of pharmacological effects that can be classified as green or white, depending on cultivation methods. However, no research has been conducted on the profiles of primary and secondary metabolites in green and white asparagus. Hence, the aim of this study was to comprehensively analyze and understand the metabolic variations associated with the color differences in asparagus under different cultivation conditions. Through extensive profiling of primary and secondary metabolites, we identified 48 hydrophilic compounds, nine policosanols, seven terpenoids, six flavonoids, and one phenolic acid. Multivariate and metabolic pathway analyses revealed notable distinctions between green and white asparagus. Green asparagus contained high levels of flavonoids, policosanols, and primary metabolites (organic and amino acids). In contrast, white asparagus contained high levels of sugars, proline, and terpenoids (tocopherols, phytosterols, and protodioscin). These results suggest that green asparagus has higher energy and amino acid synthesis metabolism for plant development and growth through photosynthesis under light cultivation conditions than white asparagus. In contrast, white asparagus accumulated stress-related metabolites, such as sucrose, proline, and phenylalanine, under dark cultivation conditions. In addition, flavonoid metabolism was activated under light conditions, whereas terpenoid biosynthesis was activated under dark conditions. The DPPH and ABTS scavenging activities of green asparagus were at least twice as high as those of white asparagus. Overall, green asparagus is a good source of nutrients and contains more antioxidants than white asparagus. These findings might be the first report of comprehensive metabolome in asparagus, and they will significantly contribute to understanding the effects of different cultivation conditions on the asparagus metabolome.

Keywords

asparagus, metabolomics, metabolites, flavonoids, rutin, terpenoids, protodioscin, antioxidant

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Introduction

Asparagus (*Asparagus officinalis* L.) is a perennial plant and food crop mainly used in the form of a thick shoot called a spear. It has been cultivated for a long time in human history and was also cultivated in ancient Egypt, Greece, and Rome and used for medicinal and edible purposes.¹ It is currently used in a variety of foods, such as salads and vegetable soups, and is among the top 10 most popular vegetables.² Asparagus is divided into green, white, purple-green, purple-blue, and pink, depending on the color difference, but it can also be divided into green and white.³ Green asparagus is popularly consumed, but white asparagus varieties are more familiar or preferred in some countries, such as the Netherlands, Belgium, and Peru.¹

These phenotypes are divided into groups based on the cultivation method, and the metabolite composition of each group

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Metabolic Profiling in Plasma and Brain Induced by 17 β -Estradiol Supplementation in Ovariectomized Mice

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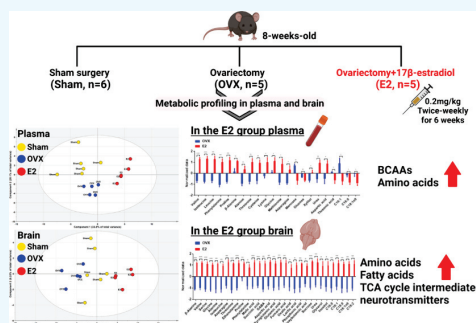


Article Recommendations



Supporting Information

ABSTRACT: 17 β -Estradiol is an ovarian hormone that regulates energy circulation and storage by acting on the central nervous system. However, the metabolic differences between the blood and brain when stimulated by 17 β -estradiol are poorly understood. Moreover, research using menopause-induced models to investigate primary metabolites in the blood and brain is limited. Thus, this study aimed to identify metabolic changes in the plasma and brain resulting from 17 β -estradiol supplementation in an estrogen-deficient mouse model. Three groups of mice were utilized: sham-operated mice (Sham), ovariectomized mice (OVX), and ovariectomized mice that received a weekly supplementation of 17 β -estradiol (E2). Plasma and brain samples from these mice were subjected to metabolic analysis using gas chromatography–time-of-flight–mass spectrometry. Compared with the plasma samples from the Sham and OVX groups, the plasma samples from the E2 group contained higher contents of branched-chain amino acids (BCAAs), such as valine, isoleucine, and leucine. Meanwhile, the brain samples from the E2 group contained higher contents of most metabolites, including BCAAs, neurotransmitters, tricarboxylic acid cycle intermediates, and fatty acids, than those from the two other groups. This study is the first to reveal differences in energy metabolism induced by 17 β -estradiol supplementation through brain metabolic profiling of ovariectomized mice, emphasizing the importance of brain metabolic profiling in menopausal hormone research.



1. INTRODUCTION

Estrogen is a vital mediator of various physiological processes in the nervous, immune, and cardiovascular systems of the body.¹ Estrogen deficiency is common in women during menopause and is associated with conditions such as osteoporosis, metabolic dysfunction, and neurological diseases.^{2,3} 17 β -Estradiol, one of the primary estrogens produced in the ovaries, regulates energy homeostasis and is closely linked to health issues in postmenopausal women.^{4,5} Historically, 17 β -estradiol has been widely used in menopausal hormone therapy (MHT) to prevent and manage these conditions. However, in recent years, the need for comprehensive studies on MHT has become clear owing to a better understanding of the timing, method, and age-dependent side effects of its administration.^{6,7} Human menopause typically spans 4–5 years and is challenging to study because of the diversity of environmental and genetic factors. Therefore, rodents, which share certain characteristics with humans and have shorter lifespans, are suitable models for studying menopause. Specifically, the ovariectomized (OVX) mouse model is valuable for researching and comprehending the hormonal changes resulting from menopause because it surgically induces estrogen deficiency.⁸

Metabolomics can explain biological changes through analysis of the concentration and pathways of metabolites that act as reactants and products in metabolic reactions.⁹

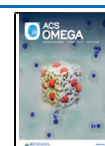
Hormones transported to the brain via the bloodstream play essential roles in various bodily functions, including the regulation of energy metabolism and immune responses.¹⁰ The blood–brain barrier (BBB) is the most tightly regulated interface among the brain and circulatory system. The BBB, which is composed of the lipophilic membrane, selectively allows hydrophilic substances transported in the blood to maintain homeostasis in the central nervous system (CNS) environment.^{11,12} In particular, 17 β -estradiol is transported through the blood–brain barrier (BBB) to the hypothalamus of the brain, where it interacts with various neurons to regulate metabolism.¹³ Therefore, it is noteworthy that 17 β -estradiol passes through the BBB and affects the central nervous system. This is why it is important to understand the metabolic differences between blood and the brain affected by 17 β -estradiol. However, little is known about the effects of 17 β -estradiol supplementation on brain metabolism.

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Effects of light-emitting diodes on the morphology and accumulation of glucosinolates, carotenoids and phenolic acids in red kale sprouts

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ABSTRACT

Kale (*Brassica oleracea* var. *acephala*) has gained popularity as a nutritious and phytochemical-rich vegetable. This study has investigated the effects of three LED treatments (white, blue, and red) on the accumulation of secondary metabolites in kale sprouts. Ten DAS, the kale sprouts were harvested, and growth measurements were measured. Furthermore, selected sprouts were stored at -80 °C for further biochemical analysis, namely, phenolic acids, glucosinolates, and carotenoids. Sprouts irradiated with red LED light showed the best SL, RT, and FW values. For total GSLs, we found that kale sprouts irradiated with white LED lights showed the best results (41.59±0.41 µmol/g DW). Among the aliphatic GSLs, we found that progoin presented the best results under blue LED light (17.93±0.49 µmol/g DW), and among the indolic GSLs, glucobrassicin showed the best results under white and red LED light. The highest concentration of total carotenoids was found in kale sprouts under white LED light exposure (3341.27±206.96 µg/g DW). Individually, β-carotene was observed in high concentration under white LED light (1935.13±87.21 µg/g DW). Among the PAs, chlorogenic acid was found in the highest concentration in the treatments under white LED light (45.78±0.73 µg/g DW). In general, kale sprouts irradiated with white LED light showed high contents of GSLs, carotenoids, and PAs. Regarding morphological characteristics, kale sprouts under red LED light showed the most promise. This research offers a valuable approach to enhancing the phytochemicals found in kale sprouting.

KEYWORDS: Kale sprouts, LED irradiation, Phytochemical composition, Secondary metabolites, HPLC

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INTRODUCTION

Kale (*Brassica oleracea* var. *acephala*) is a cool-season crop from the Brassicaceae family. Its leaves, rich in vitamins and essential mineral components, are commonly used alone or together for human and animal feed (Life, 2024). In recent years, kale has gained the scientific community's attention due to its nutritional composition (Ware, 2020), and consumer acceptance (Adeyeye *et al.*, 2018). In addition, due to its high content of bioactive compounds such as vitamins (Becerra-Moreno *et al.*, 2014), glucosinolates (Bhandari *et al.*, 2015; Liu *et al.*, 2022), flavonoids (Lännenpää, 2014; Panche *et al.*, 2016), phenolics (Ayaz *et al.*, 2008; Cartea *et al.*, 2011; Bianchi *et al.*, 2024),

micro and minerals (Sikora & Bodziarczyk, 2013; Thavarajah *et al.*, 2016). Notwithstanding, kale has been used in traditional medicinal systems to treat several diseases, including diabetes, rheumatism, and hepatic diseases (Raiola *et al.*, 2017; Chen *et al.*, 2018; Šamec *et al.*, 2019; Luang-In *et al.*, 2020).

A sprout is a young plant that has germinated seeds. There are several varieties of them and they are usually eaten raw (Healthline, 2024). Unsurprisingly, sprouts are nutrient-dense and contain beneficial plant compounds despite their low-calorie content. Depending on the variety, they contain different amounts of vitamins and minerals (Chang *et al.*, 2019). Nutrient levels in sprouted grains, legumes, vegetables, and seeds tend to

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Optimizing suitable solvent for phenylpropanoid extraction and antioxidant activities in *Agastache rugosa* hairy roots

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ABSTRACT

Agrobacterium-mediated hairy roots (HRs) can induce genetic stability, rapid growth, and the synthesis of bioactive compounds in plant roots. *Agastache rugosa* is otherwise called Korea Mint and several studies have reported that this plant has been used for the treatment of various diseases due to the presence of a variety of bioactive compounds. *A. rugosa* HRs are rich in secondary metabolites than the seedling roots, and the HRs extract might be more useful in pharmacology, especially in cosmetology. This study aimed to select the suitable solvent for the extraction of phenylpropanoid compounds, total phenolic (TP), flavonoid (TF), and antioxidant activities (DPPH, ABTS scavenging activity, and reducing power assay). In this study, we extracted the *A. rugosa* HRs with three different extracts of solvent (water, MeOH, and EtOH) of *A. rugosa* HRs and analyzed the phenylpropanoid compounds, TP, TF, and antioxidant activities. The result showed that 70% MeOH extracts showed the highest activities in all assays, followed by the 70% EtOH, and water extracts. In addition, 70% of MeOH extracts showed the highest TP and TF (46.14 ± 0.25 GAE mg/g DW and 65.46 ± 1.41 QE mg/g DW, respectively) contents, which was 1.96- and 1.76- times higher than that of the water extracts. The phenylpropanoids in *A. rugosa* HR extracts were identified by using HPLC, and the results showed that 70% MeOH and EtOH showed the highest contents. Regarding these results, we can conclude that 70% MeOH is the optimal solvent to extract the *A. rugosa* HRs for the highest phenylpropanoid, TP, TF content, and antioxidant activities. This study might be useful for producing useful compounds at an industrial scale.

KEYWORDS: *Agastache rugosa*, Extraction of different solvents, Antioxidants, Phenolics, Flavonoids, Phenylpropanoids

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INTRODUCTION

Oxidative processes are crucial for both physiological functions in living organisms and the preservation of food, with oxidation being vital for cellular life. However, the free radicals and reactive oxygen species (ROS) generated during these processes can pose detrimental effects on health by damaging cells and impacting cellular signaling pathways (Bae *et al.*, 1999; Antolovich *et al.*, 2002). Additionally, these radicals contribute to the chemical degradation of food, emphasizing the importance of understanding oxidative mechanisms (Colbert & Decker, 1991). Natural antioxidants, including phenolics and flavonoids found

in a diverse array of plant-based foods such as fruits, vegetables, cereals, and spices, offer protective roles against oxidative stress (Shahidi & Zhong, 2015). These antioxidants can be extracted and utilized in pharmacological and nutraceutical applications due to their health benefits, contrasting with synthetic antioxidants that have been linked to adverse effects, leading to a shift towards natural alternatives (Choe & Yang, 1982; Antolovich *et al.*, 2002; Shahidi & Zhong, 2015).

The increased interest in natural antioxidants over synthetic ones underscores the exploration of plant-based sources for new antioxidant compounds, driven by their potential

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Letter to the editor:

RECENT INSIGHTS INTO LUTEOLIN AND ITS BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES

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Luteolin (LUT), or 3',4',5,7-tetrahydroxyflavone, is a flavonoid generally found in glycosylated form in a wide range of plants such as medicinal herbs, fruits and vegetables (Arampatzis et al., 2023). Chinese traditional medicine makes extensive use of LUT to treat numerous conditions, particularly inflammatory disorders, hypertension, and cancer (Lin et al., 2008). According to IUPAC chemical nomenclature, LUT is named 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one. This compound was first isolated in its pure form in 1829 by a French chemist Michel Eugène Chevreul (Jain and Tiwari, 2020).

LUT exerts a range of beneficial effects on human health, with the following being reported in many studies: antiallergic, anti-inflammatory, antidiabetic, neuroprotective, and anticancer. Due to their chemical nature, LUT and its glycosides also display antioxidant properties, scavenging free radicals derived from oxidation and chelating metal ions (Cai et al., 1997; Choi et al., 2007; Muruganathan et al., 2022). In recent years, LUT has attracted much attention from the pharmaceutical, food, and cosmetic industries for its plethora of biological and pharmacological activities. Herein, we present a summary of recent key studies performed to evaluate the biological and pharmacological activities of LUT (Table 1).

Effect of LED Lights on Secondary Metabolites and Antioxidant Activities in Red Pakchoi Baby Leaves

Leonel Tarcisio da Cristina Bungala, Sang Un Park, Bao Van Nguyen, Jinsu Lim, Kihyun Kim, Jae Kwang Kim, Chang Ha Park, Anh Tuan Le, Yong Suk Chung,* and Hyeon Ji Yeo*



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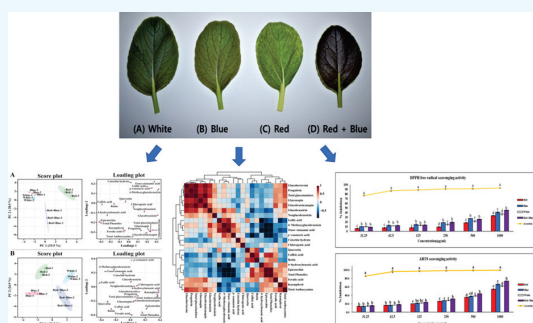


Article Recommendations



Supporting Information

ABSTRACT: Pakchoi (*Brassica rapa* subsp. *chinensis*) is one of the most widely consumed vegetables in Asian countries, and it is high in secondary metabolites. The availability, quantity, and quality of light play a critical role in the growth and development of plants. In this study, we investigated the effect of LEDs (light-emitting diodes; white, blue, red, and red + blue) on anthocyanin, glucosinolates, and phenolic levels in red pakchoi baby leaves. On the 24th day after sowing (DAS), red baby pakchoi leaves were harvested, and shoot length, root length, and fresh weight were measured. Among the different LED treatments, there was no significant difference in shoot length, whereas the highest root length was achieved in the red + blue LED treatment (23.8 cm). The fresh weight also showed a significant difference among the different LED treatments. In total, 12 phenolic and 7 glucosinolate individual compounds were identified using high-performance liquid chromatography (HPLC) analysis. The highest total glucosinolate (2937 $\mu\text{g/g}$ dry wt) and phenolic (1589 $\mu\text{g/g}$ dry wt) contents were achieved in baby leaves exposed to red + blue light. Similarly, the highest contents of total anthocyanins (1726 $\mu\text{g/g}$ dry wt), flavonoids (4920 $\mu\text{g/g}$ dry wt), and phenolics (5900 $\mu\text{g/g}$ dry wt) were achieved in the red + blue treatment. Plants exposed to red + blue LED light showed the highest accumulation of anthocyanin, glucosinolates, and phenolic compounds. For antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazylradical) free radical scavenging, ABTS (2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid) radical scavenging, and reducing power assays were performed, and the antioxidant activity of red pakchoi baby leaves grown under red + blue LED light was found to be the best. The metabolic profiling of the identified metabolites revealed distinct separation based on the secondary metabolites. This research will be helpful for farmers to choose the best LED light combination to increase the secondary metabolic content in pakchoi plants.



1. INTRODUCTION

Pakchoi, also known as Chinese cabbage (*Brassica rapa* subsp. *chinensis*), belongs to the Brassicaceae family. Because of its beneficial phytochemical composition, it is grown and consumed annually in most Asian countries.¹ It is grown and consumed throughout the year because it has numerous health-promoting compounds, such as anthocyanins, carotenoids, chlorophylls, flavonoids, hydroxycinnamic acid derivatives, isorhamnetin, kaempferol, phenolics, quercetin, vitamins, and minerals.^{2,3}

Glucosinolates are a large group of plant secondary metabolites with nutritional effects and biological activity. Glucosinolates are primarily found in cruciferous plants, including the widely consumed Brassicaceae family.⁴ One of the reasons for the high consumption of cruciferous vegetables is that the breakdown products of glucosinolates, such as nitriles, oxazolidines, thiocyanates, isothiocyanates, and epithionitriles, are effective in reducing the risk of cancer and heart failure in humans.^{5,6} One of the most important groups of phytochemicals with antioxidant capacity is phenolic

compounds.⁷ The phenolic content of Brassica vegetables has recently been examined. Despite variations among species and even harvests from the same species, these crops are usually rich in phenolic compound composition.⁸

Among the various environmental factors, light quality is crucial for photosynthesis, plant growth, and development.⁹ In plants, light-emitting diode (LED) affects metabolites in a significant way and plays an important role in their physiology.¹⁰ Furthermore, LED lights are more effective than fluorescent lights for nonheading pakchoi reproductive and vegetative growth. Blue LED light promotes vegetative growth, whereas red LED light promotes reproductive growth.¹¹ Several studies have reported that different LEDs

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Comparative Analysis of Primary and Secondary Metabolites in Different In Vitro Tissues of *Narcissus tazetta* var. *chinensis*

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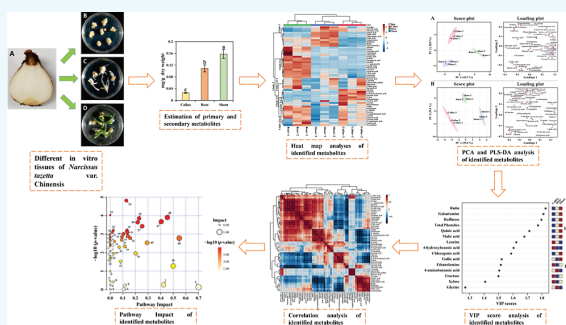


Article Recommendations



Supporting Information

ABSTRACT: *Narcissus tazetta* var. *chinensis* is a perennial monocot plant that is well known for its pharmaceutical and ornamental uses. This study aimed to understand the changes in the primary and secondary metabolites in different in vitro tissues of *N. tazetta* (callus, adventitious root, and shoot) using high-performance liquid chromatography and gas chromatography time-of-flight mass spectrometry. In addition, to optimize the most efficient in vitro culture methods for primary and secondary metabolite production, *N. tazetta* bulbs were used as explants and cultivated in Murashige and Skoog (MS) medium containing different hormones at various concentrations. In addition, the present study found suitable hormonal concentrations for callus, adventitious root, and shoot induction and analyzed the primary and secondary metabolites. The MS medium supplemented with 1.0 mg L⁻¹ dicamba, 3.0 mg L⁻¹ indole-3-butyric acid (IBA), and 3.0 mg L⁻¹ 6-benzylaminopurine (BAP) was the most efficient media for callus, adventitious root, and shoot induction in *N. tazetta*. The tissue induced in this medium was subjected to primary (amines, amino acids, organic acids, sugars, and sugar alcohols) and secondary metabolite (galantamine and phenolic acids) analysis. The shoots and roots showed the highest amounts of metabolites. This study showed that bulb in vitro culture can be an efficient micropropagation method for *N. tazetta* and the production of primary and secondary metabolites, offering implications for the mass production of primary and secondary metabolite compounds from *N. tazetta* tissues generated in vitro.



INTRODUCTION

Narcissus tazetta var. *chinensis*, a perennial monocot plant in the Amaryllidaceae family, is well-known for its pharmaceutical and ornamental uses.^{1,2} Amaryllidaceae alkaloids are produced by species of Amaryllidaceae, including *Narcissus* spp. (daffodil). Most of the alkaloids in Amaryllidaceae are known to have biological activity. All major structural classes of alkaloids found in Amaryllidaceae have biological activities.^{3,4} As a result, many alkaloids from Amaryllidaceae are used as medicines, and other alkaloids in the family have the potential to be used in pharmaceuticals.⁵

Secondary metabolites in plants include various compounds that are synthesized in numerous ways through precursors and in response to stimuli.⁶ The secondary metabolites in plants have various functions, such as attracting pollinators, repelling herbivores, and responding to environmental stress.^{7,8} Plants are an essential source for discovering novel bioactive compounds, and the metabolites found in plants are used as essential substances to make products for use in the pharmaceutical, cosmetic, and food industries.^{9,10} Plant cultivation is mainly used to harvest secondary metabolites from plants; however, the innovation of tissue culture in plant

systems has opened another path for the collection of secondary metabolites.¹¹

Galantamine is an alkaloid from the Amaryllidaceae family. It is a dibenzofuran-type base that has been found in several species of this family.^{12,13} Acetylcholinesterase inhibitor galantamine is a powerful drug used worldwide to treat the symptoms of Alzheimer-type senile dementia.^{14,15} The galantamine used in the medical field can be produced in two ways, total synthesis and extraction, using Amaryllidaceae plants.^{16,17} Phenylpropanoids comprise a broad group of organic compounds that are synthesized by a combination of the amino acids tyrosine and phenylalanine in plants.^{18,19} Previous studies have reported that phenylpropanoids have various positive benefits for human health (helps to prevent diabetes, cancer, and heart disease) and pharmaceutical sectors.^{20–22}

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RESEARCH

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Identification of metabolite changes and evaluation of biological activities in edible flowers of *Magnolia kobus* at different developmental stages

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Abstract

Background *Magnolia kobus* belongs to the Magnoliaceae family and the genus *Magnolia*. The flowers are widely used in herbal tea and dietary supplements. It is effectively used in traditional medicines and its chemical constituents have various biological activities, such as anti-obesity, antioxidant, anticancer, anti-inflammatory, and anti-hyperlipidaemic activities. The flower developmental process of *M. kobus* involves a complex regulatory network and is intensively related to the quality and relative composition of primary and secondary metabolites of the flower. Hence, the identification of metabolic changes during different developmental stages of the flower was important for enhancing organoleptic and nutritional characteristics. To our knowledge, to date, none of the studies has investigated the relationship between metabolic changes (primary and secondary metabolites) during different developmental stages of the *M. kobus* flower.

Results This study investigated metabolic changes in 47 hydrophilic and 13 lipophilic compounds and biological activities in *Magnolia kobus* flowers during four developmental stages. Metabolites were identified using HPLC, GC–qMS, and GC–TOFMS. Throughout flower development, metabolite accumulation showed significant differences based on metabolomics approaches. The dynamic changes of 21 differential metabolites occurred between the young and mature stages. Flower buds exhibited high levels of phenylpropanoids and phytosterols. The total phenolic and flavonoid contents were most abundant in the buds and decreased from the bud to the old stages. Flower bud extracts showed the most powerful effects in three antioxidant assays and antibacterial effects against 10 pathogenic bacterial strains.

Conclusions These findings emphasise the value of *M. kobus* flower buds as an edible natural source and provide valuable insight into the metabolic changes that occur in *M. kobus* flowers during development.

Keywords Edible flower, *Magnolia kobus*, Metabolomics, Phenylpropanoid, Phytosterol, Biological activity

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Comparative metabolomic analysis of mouse plasma in response to different dietary conditions

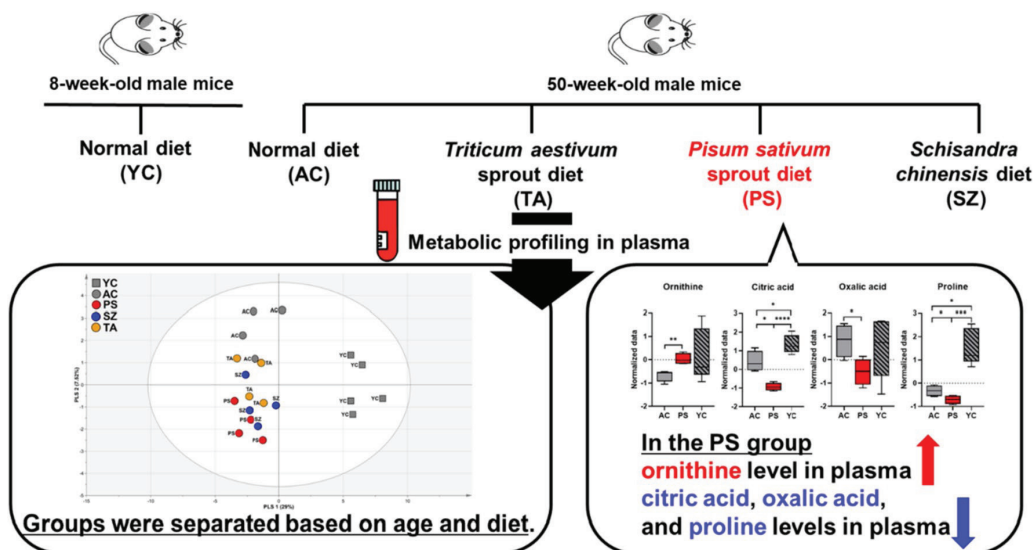
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Abstract

Plasma metabolites offer insights into aging processes and aging-related biomarkers. Here, the dietary effects of various functional foods on older adult mice were evaluated using metabolomic techniques. Fifty-week-old mice were divided into four groups (n = 4 each) and fed either a normal diet (AC) or the diets from *Triticum aestivum* sprout (TA), *Schisandra chinensis* (SZ), or *Pisum sativum* sprout (PS) extracts. Additionally, a group of 8-week-old mice fed a normal diet (YC; n = 5) was included for the comparison. The PS group had a significantly lower free fatty acid content and higher ornithine, proline, citric acid, and oxalic acid contents than the AC group. The PS group also showed reduced oxidative stress and muscle damage, suggesting the higher anti-aging efficacy of *P. sativum* sprouts than the other diets. These findings suggest plasma metabolite profiling is an effective tool to assess the anti-aging effects of functional foods.

Graphical abstract



Keywords Aging · *Pisum sativum* · *Triticum aestivum* · *Schisandra chinensis* · Metabolomics

So Hwi Yang and Ye Jin Kim contributed equally to this work as co-first authors.

Extended author information available on the last page of the article



Molecular protocol to develop β -carotene-biofortified rice events via molecular optimization

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ABSTRACT

Providing food with nutrition and functionality is crucial for sustaining human life. Rice (*Oryza sativa* L.) is a representative staple crop with high carbohydrate content but low amounts of essential amino acids, micronutrients, and carotenoids such as provitamin A. To improve the nutritional quality, rice endosperm was biofortified to accumulate carotenoids such as β -carotene through genetic engineering (i.e., using synthetic carotenoid biosynthetic genes, a nonmammalian viral polycistronic sequence, and an optimized promoter and transit peptide) and high-throughput rice transformation (approximately 300 transgenic plants per construct). To facilitate the safety assessment of genetically modified food, molecular characterization was performed to select elite lines equipped with a single intergenic insertion of T-DNA, high transgene expression, in this case leading to high carotenoid content, and with phenotypic and compositional substantial equivalence. In this study, we present β -carotene-biofortified rice event candidate lines eligible for commercial use and a disclosed molecular protocol for the development of biotech rice crops.

1. Introduction

Crop biofortification has been addressing global food, nutritional security, and the malnutrition issues resulting from the relatively low density of micronutrients in many staple crops using a range of methods including agronomic, conventional breeding, and transgenic approaches (Bhardwaj et al., 2022; Garg et al., 2018; Gebregziabher et al., 2023). Some of these essential nutrients, such as vitamins (provitamin A β -carotene, vitamin B9 folate, and vitamin C ascorbate), micronutrient minerals (zinc and iron), and essential amino acids (lysine, methionine, and tryptophan), are of relatively low density in staple crops. These crops include cassava (*Manihot esculenta*), maize (*Zea mays*, corn), plantain (*Musa acuminata* $\times$ *Musa balbisiana*), potato (*Solanum tuberosum*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), soybean (*Glycine max*), sweet potato (*Ipomoea batatas*), and wheat (*Triticum aestivum*). According to the HarvestPlus Annual Report (HarvestPlus, 2020), 435 biofortified varieties of 12 crops were released in >30 countries and are

being grown in >63 countries, mainly with three nutrients: β -carotene in sweet potato, cassava, maize, and banana; iron in beans, lentil, cowpea, and pearl millet; and zinc in sorghum, maize, rice, and wheat. Most successful implementation has been achieved via conventional breeding of β -carotene-rich, orange-fleshed sweet potatoes, which were released with the largest number of 143 varieties in African, Asian, Latin American, and Caribbean countries in 2019. This implementation, supporting the nutritional significance of β -carotene, won the World Food Prize in 2016 (Gebregziabher et al., 2023; International Potato Center, 2019).

Likewise, breeding methods have become much more practical in the direct use of biofortified crops; however, the use of transgenic methods, known as genetically modified (GM) technology, has been unavoidable when the genetic diversity for the target traits is limited in the host crops (Garg et al., 2018). Historically, crop biofortification via GM technology began with Golden Rice (GR) 1 through the overexpression of two β -carotene biosynthetic genes, *NpPsy* and *PaCrtI*, which encode phytoene synthase (PSY) from daffodil (*Narcissus pseudonarcissus*) and

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Article

Enhancement of Phenylpropanoid Accumulation and Antioxidant Activities of *Agastache rugosa* Transgenic Hairy Root Cultures by Overexpressing the Maize *Lc* Transcription Factor

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Abstract: *Agastache rugosa* is also known as Korean mint, and it has numerous health benefits due to its rich source of phenolic compounds. The main objective of this study was to produce a *ZmLC*-overexpressing transgenic hairy root line via *Agrobacterium rhizogenes*-mediated transformation. The overexpressing transgenic lines were screened using qRT-PCR after exposure to light conditions. The best hairy root line was selected, and the expression levels of phenylpropanoid biosynthetic pathway genes and phenylpropanoid compound accumulation were analysed using qRT-PCR and HPLC, respectively. In addition, antioxidant activities (RPA, ABTS, and DPPH), total phenolic content, and total flavonoid content were analysed. The *ZmLC*-overexpressing transgenic line upregulated all the phenylpropanoid pathway genes, which led to the higher accumulation of phenylpropanoid compounds in the transgenic line than in the control line. In addition, the total phenolic and flavonoid content was significantly higher in the transgenic line. The antioxidant activity assay showed that the transgenic hairy root line had significantly higher activity than that of the control lines. Thus, *ZmLC* positively enhances the phenylpropanoid biosynthetic pathway and antioxidant activities in *A. rugosa*. The results show that *ZmLC* can be used to enhance phenylpropanoid compounds and antioxidant activities in transgenic *A. rugosa* hairy root lines via the genetic engineering approach.

Keywords: *Agastache rugosa*; Korean mint; transcription factor; *ZmLC*; phenolic compound; antioxidant activities

1. Introduction

Agastache rugosa is a precious medicinal plant from the Lamiaceae family that is grown in many Korean and East Asian areas. *Agastache rugosa* is also a famous plant in traditional Chinese medicine and is included in the list of fifty basic herbs [1]. It is a plant source containing both bioactive compounds [2] used to treat anxiety, cholera, infections, nausea, and gastrointestinal problems [3,4], and antimicrobial and antifungal agents [4]. It

RESEARCH

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Integrated metabolome and transcriptome analyses provide comprehensive insight into dark- and light-responsive mechanisms in *Althaea officinalis* hairy root cultures

Yun Ji Park^{1†}, Jae Kwang Kim^{2,3†}, Nam Su Kim⁴, Young Jin Park², Ramaraj Sathasivam^{5*} and Sang Un Park^{5,6*}

Abstract

Background Light, an essential factor in plant development, exerts a significant impact on both primary and secondary metabolism in plants. *Althaea officinalis*, commonly known as marshmallow, offers versatile applications through its leaves and roots. With a plethora of identified bioactive compounds and their extensive use in food, health, and supplements, it is widely cultivated globally. This study aimed to demonstrate the definitive positive impact of dark and light irradiation on both primary and secondary metabolite production in *A. officinalis* hairy roots and to elucidate the light-responsive mechanism through integrated metabolome and transcriptome analysis.

Results When exposed to light, significant changes with a greenish colour shift were observed in 60 metabolites. Multivariate statistical analysis revealed a distinct separation between light- and dark-treated hairy roots, likely attributed to metabolites such as glutamic acid, phenylalanine, catechin hydrate, and chlorophyll. Correspondingly, the pathways significantly impacted included galactose metabolism, alanine, aspartate, and glutamate metabolism, flavone and flavonol biosynthesis, and phenylalanine metabolism. Light-responsive differentially expressed genes associated with pigment and phenylpropanoid biosynthetic pathways were analysed and compared via RNA sequencing. Furthermore, among the light-related transcription factors, including CONSTANS-LIKE and double B-box zinc finger, which are responsible for photomorphogenic modulation, were upregulated. Moreover, light-responsive genes, such as ribulose biphosphate carboxylase, photosystem II, and chlorophyll A-B binding family protein, were upregulated.

Conclusions These findings emphasise that exposure of *A. officinalis* hairy root culture to light conditions is a useful method for enhancing most of the primary and secondary metabolites.

Keywords *Althaea officinalis*, Light irradiation, Metabolome, Transcriptome, Hairy root culture

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RESEARCH ARTICLE

Effect of different NaCl concentrations on secondary metabolites and antioxidant activity in three radish (*Raphanus sativus* L.) varieties

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Abstract

Salinity, a critical environmental stressor, imposes constraints on field crops' anatomical structure, growth, and physiology. This study has uniquely evaluated the effect of 0 (control), 100, and 200 mM NaCl on phenolic acids (PAs), total anthocyanin content (TAC), chlorophyll, and antioxidant activity in three radish varieties and contributes novel insights to the scientific community. The salt concentration significantly influenced shoot length (SL), root length (RL), and fresh weight (FW) in radish sprouts. The varying salt concentrations did not affect *chlorophyll a*, but *chlorophyll b* and total chlorophyll content (TCC) increased the red and super red varieties. The total phenolic content (TPC) and total flavonoid content (TFC) registered a slight increase in some varieties under 100 mM salt concentration, with the highest accumulation of these secondary metabolites found in the super red variety. Five individual PAs were identified using high-performance liquid chromatography (HPLC) analysis, with salt treatment significantly affecting ferulic acid and *trans*-cinnamic acid concentrations. The lowest level of anthocyanin was found in all green varieties. Salinity stress of 100 and 200 mM affected the TAC in the red and super red varieties compared to the control. Furthermore, among the three varieties treated with NaCl, the super red IC₅₀ values displayed the highest 2,2,1-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) free radical scavenging activity compared to the other treatments. These results suggest that a 100 mM NaCl concentration can be used as an inducer to improve the accumulation of phytochemicals in the radish seedlings 12 days after sowing (DAS).

Keywords

Radish cultivars; salt stress; concentration; phytochemicals

Introduction

Radish (*Raphanus sativus* L.) is an important root-grown vegetable consumed worldwide because of its nutritional content and benefits to human health and well-being (1). Recent studies on the nutritional properties of radish sprouts show that consumers are becoming more inclined towards consuming this type of food due to their high content of phytochemicals (2). However, the crop faces losses due to the various abiotic stresses.



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Effect of high-temperature stress on phenylpropanoid biosynthesis and antioxidant activities of *Acer palmatum*

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ABSTRACT

Acer palmatum is an ornamental tree grown in East Asia and is exposed to the possible hazards of global climate changes and increased temperatures. In this context, a study was conducted in a greenhouse at the experimental farm of Chungnam National University, Daejeon, South Korea from April to August 2023, to explore the consequences of high temperature stress on *A. palmatum* and assess the phenylpropanoid biosynthesis, antioxidant activity, and chlorophyll content in its leaves. One-year-old seedlings were treated at 40 °C for 1-3 days to determine the phenolic and flavonoid content, chlorophyll concentrations, and antioxidant activity at various intervals. The highest phenylpropanoid, total phenolic compound, and total flavonoid contents were observed during the first 2 days of high temperature treatment and reduced afterwards. Regarding the duration of the plant's exposure, 1 day of exposure was associated with the highest antioxidant level assessed with DPPH and ABTS assays, while antioxidant activity was lowest under a long duration. The chlorophyll B content increased 2-fold with 1 day of heating exposure, in contrast to the levels of chlorophyll A and B. However, *A. palmatum* benefitted from a moderate level of heat stress, which stimulated the development of protective biochemical responses without developmental features. At the same time, extended exposure compromised the physiological health of the plant, reducing the phenolic content, antioxidant activity, and chlorophyll content.

KEYWORDS: *Acer palmatum*, Phenolic compounds, Flavonoids, Antioxidant activities

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INTRODUCTION

The palmate maple (*Acer palmatum*), a deciduous tree belonging to the Sapindaceae family, is well-known for its vibrant red leaves in the fall. Trees from the *Acer* genus are widely distributed, particularly in East Asia, North America, and Europe (Sun *et al.*, 2022). *A. palmatum* is an ornamental deciduous tree that is famous for its colourful foliage and extensively cultivated in Japan, Korea, and China (Wan *et al.*, 2023). The leaves of *A. palmatum* contain chlorophyll and accessory pigments, such as carotenoids and anthocyanins. However, these pigments remain hidden by chlorophyll during the spring and summer. As days shorten and temperatures drop in the fall, chlorophyll production ceases, and the existing chlorophyll decomposes. An abscission layer forms between the leaves and branches, allowing the leaves to fall off in the

wind. Since starch cannot be transported to the stem due to the abscission layer, it accumulates in the leaves, eventually causing chlorophyll degradation. Pigments, such as carotenoids, then become visible, leading to the characteristic red colour of the leaves (Primka & Smith, 2019).

Global warming has led to rising temperatures each year, making high-temperature stress a significant factor that negatively affects plant development and growth. High temperatures disrupt photosystem II, reduce photosynthetic and respiratory activities, and diminish photosynthetic pigments (Elnaggar *et al.*, 2024). They also lead to metabolic imbalances, oxidative damage, and ultimately plant wilting and death (Haider *et al.*, 2021). Plants respond to heat stress by undergoing various physiological and biochemical changes. High temperatures disturb plant cell homeostasis, ion distribution, and osmotic

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ORIGINAL ARTICLE



Metabolic profiling characterizes the genetic diversity of genetically modified and conventional rice

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Abstract

Three genetically modified (GM) rice varieties were developed: drought-tolerant, insect-resistant, and marker-free transgenic Bt rice. The metabolomic approaches were applied to the seeds and leaves of three GM rice plants to comparatively investigate the effects of genetic modification on GM and non-GM rice. Among the non-GM rice, commercial rice consisting of *japonica*, *indica*, and *japonica* × *indica* (hybrid) subspecies was grown in a greenhouse together with GM rice. Multivariate analysis revealed that natural genotypes (*japonica*, *indica*, and hybrids) had a much stronger effect on rice seed and leave compositions than genetic modifications. Compared to *japonica* rice, raffinose and inositol were higher in the seeds and leaves of *indica* and hybrid rice, suggesting a metabolic phenotype for the genetic diversity of subspecies with different origins. Our results demonstrated that metabolomics approaches can be used to evaluate the unintended effects of GM and non-GM rice ingredients. Furthermore, metabolic profiling reflects the natural variability of the metabolites associated with their origins.

Keywords Metabolomics · Genetically modified rice · *Japonica* · *Indica* · Seed · Leaf

Introduction

As the world's population grows, agricultural production and quality have become crucial regarding meeting the increasing food demand. Improved cultivars and increased use of fertilizers and pesticides have played important roles in increasing global food production; however, these aspects are accompanied by detrimental effects (Kobayashi et al.

2023; Dutta et al. 2016). Therefore, genetically modified (GM) crops have become relevant as a way to achieve higher yields and improved quality, as well as promoting eco-friendly agriculture (Dutta et al. 2016). GM crops were first introduced in 1996. GM crops are engineered to improve their traits by inserting targeted DNA fragments into the host genome, leading to improved crop quality and insect and herbicide resistance (Alikord et al. 2022; Klümper and Qaim 2014).

Rice plants (*Oryza sativa* L.) contain useful nutrients such as minerals, vitamins, phytochemicals, essential amino acids, and fatty acids (Birla et al. 2017). Therefore, rice is an important grain and essential food supplied to more than half of the world's population and Asian countries (Kobayashi et al. 2023). Genetic engineering techniques increase resistance to herbicides and pesticides and increase essential protein and nutrient content by promoting photosynthesis (Fraiture et al. 2016). Despite the advantages of GM crops, environmental and health concerns regarding their use have been raised. Therefore, studies on the safety of GM crops are required to determine their potential adverse effects (Alikord et al. 2022). To ensure GM crop safety, comprehensive analyses of their nutritional components, allergenicity, and cytotoxicity are being conducted to compare GM crops or products with their parent or commercial varieties

Ye Jin Kim and Sung-Dug Oh have contributed equally to this study.

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Research Paper

Polyporus ulleungus mycelia cultured in MEB medium produce metabolites with anticancer property

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Abstract

Cancer cells are characterized by apoptosis evasion and uncontrolled cell cycle progression. To combat these characteristics, efforts have been made to find novel natural-source anticancer compounds. The aim of this work is to find new anticancer compounds in *Polyporus ulleungus* (*P. ulleungus*) mycelial culture extracts. *P. ulleungus* mycelium was cultured on four individual media (DYB, MEB, MYB, and PDB) and four extracts were generated from the mycelium culture media. Extracts of *P. ulleungus* mycelium cultured in MEB medium (pu-MEB) significantly reduced cancer cell growth by triggering apoptosis and S phase arrest. Furthermore, the anticancer effects of pu-MEB were not confined to one type of cancer. Taken together, our results confirmed that *P. ulleungus* mycelia cultured in MEB medium produce metabolites that exhibit anticancer properties. Development of an optimal medium for *P. ulleungus* mycelium through optimization of medium components will enable *P. ulleungus* mycelium to produce metabolites with more anticancer efficacy.

Keywords: *Polyporus ulleungus*, mycelium culture extract, drug screening

Introduction

Mushrooms are not only a source of nutrients but are also recognized as health-promoting foods as they produce physiologically active substances [1]. Mushrooms synthesize primary metabolites that are essential for mushroom growth and secondary metabolites that are produced in response to various environmental stimuli [2]. Secondary metabolites exhibit immunomodulatory, anticancer, antibacterial, anti-HIV, and antidiabetic properties [2, 3]. Among them, the anticancer properties of mushrooms are receiving considerable attention, and clinical trials to evaluate the effectiveness of mushrooms are increasing [4]. The 5-year survival rate of non-small cell lung cancer patients treated with reishi mushrooms increased, proving the efficacy of reishi mushrooms [5]. Additionally, *Agaricus bisporus* has

proven efficacy in treating patients with recurrent prostate cancer by inhibiting prostate-specific antigen [6]. Therefore, identification of new mushrooms with anticancer property and clinical application of their efficacy will become a new treatment method.

Mushrooms are organisms characterized by a life cycle consisting of two distinct stages: mycelium and fruiting body [7]. Mycelium is a thread-like organism that grows in soil, wood, or other substrates and absorbs nutrients [7]. The fruiting body is the part of the mushroom that can be found above ground and consists of a cap, stem, gills or stomata, depending on the species [7]. Comparison of the composition and concentration of various compounds in fruiting bodies and mycelium revealed that mycelium has a higher content of some bioactive compounds than the

Altered Metabolic Phenotypes and Hypothalamic Neuronal Activity Triggered by Sodium-Glucose Cotransporter 2 Inhibition (*Diabetes Metab J* 2023;47: 784-95)

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We greatly appreciated the insightful comments on our recently published paper, “Altered metabolic phenotypes and hypothalamic neuronal activity triggered by sodium-glucose cotransporter 2 inhibition [1].”

First, we would like to address the concerns raised. Specifically, we acknowledge the absence of comprehensive metabolic characterization, especially in mice subjected to a high-fat diet. You correctly pointed out the importance of metabolic traits such as glycemic status and insulin resistance when studying the effects of drugs like sodium-glucose cotransporter 2 (SGLT2) inhibitors [2]. We agree that providing a more detailed metabolic profile of the mice, including parameters like glucose metabolism and insulin sensitivity, would add valuable context to our findings. While these specific results were not included in the manuscript, we did confirm a reduction in plasma glucose level in mice treated with dapagliflozin.

In our future research, we will consider incorporating these variables to gain a more comprehensive understanding of the

metabolic effects of SGLT2 inhibitors under various dietary conditions.

Second, we understand the concerns raised about whether the impact of dapagliflozin on appetite, energy expenditure, and body weight is a direct result or an indirect effect. We acknowledge the complexity of drug interactions, including both direct and indirect influences, which can affect outcomes in clinical and preclinical studies. While investigating the precise mechanisms by which dapagliflozin affects the central nervous system may not be the primary clinical objective, such research can contribute to our understanding of the drug's actions and its potential optimization in combination with other therapies. For instance, the hyperphagic effect of SGLT-2 inhibitor treatment may be beneficial in conjunction with glucagon-like peptide-1 receptor agonist administration, which leads to a reduction in appetite, for the treatment of obesity [3,4]. In our future studies, we intend to thoroughly examine these mechanisms, as you suggested, to better inform treatment strategies for indi-

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RESEARCH ARTICLE

Minimally Invasive Syringe-Injectable Hydrogel with Angiogenic Factors for Ischemic Stroke Treatment

Donggue Kim, Ji Woo Lee, Yang Tae Kim, Junhyeok Choe, Gaeun Kim, Chang Man Ha, Jae Geun Kim,* Kwang Hoon Song,* and Sunggu Yang*

Ischemic stroke (IS) accounts for most stroke incidents and causes intractable damage to brain tissue. This condition manifests as diverse aftereffects, such as motor impairment, emotional disturbances, and dementia. However, a fundamental approach to curing IS remains unclear. This study proposes a novel approach for treating IS by employing minimally invasive and injectable jammed gelatin-norbornene nanofibrous hydrogels (GNF) infused with growth factors (GFs). The developed GNF/GF hydrogels are administered to the motor cortex of a rat IS model to evaluate their therapeutic effects on IS-induced motor dysfunction. GNFs mimic a natural fibrous extracellular matrix architecture and can be precisely injected into a targeted brain area. The syringe-injectable jammed nanofibrous hydrogel system increased angiogenesis, inflammation, and sensorimotor function in the IS-affected brain. For clinical applications, the biocompatible GNF hydrogel has the potential to efficiently load disease-specific drugs, enabling targeted therapy for treating a wide range of neurological diseases.

1. Introduction

Ischemic stroke (IS), which constitutes the majority of stroke cases, occurs mainly because of the blockage of blood vessels toward the brain, leading to the depletion of oxygen and glucose, and subsequent cell death.^[1] This shortage of blood supply to the brain can eventually lead to various aftereffects, such as sensorimotor impairment, speech and communication deficits, memory impairment, emotion processing deficits, and mental disorders. In particular, ≈50% of survivors of IS suffer from sensorimotor deficits.^[2] Furthermore, IS often accompanied by various complications, such as cardiac complications, pneumonia, venous thromboembolism, fever, pain, dysphagia, incontinence, and depression, all of which affect patients' daily lives

and impede neurological recovery.^[3] Currently, the clinical treatment of IS primarily involves the restoration of blood flow to the brain. For example, intravenous thrombolytics are commonly used as IS treatments to dissolve clots that block blood vessels. An endovascular procedure using catheters and stents to physically remove blood clots is an alternative approach.^[4] However, conventional approaches require swift action following the onset of stroke and merely offer temporary solutions with a high risk of recurrence. In addition, they may not be able to support the regeneration of brain tissues already damaged by stroke. Therefore, there is a growing need to develop an effective IS treatment that can fundamentally restore the damaged brain following its aftereffects.^[1,2]

Pharmacological approaches are the current mainstay treatments for IS,^[5,6] and various strategies have been developed to deliver appropriate drugs to target brain tissues. Drugs injected into the bloodstream may not be able to approach the target brain tissues because the blood–brain barrier (BBB) hinders the extravasation of drugs.^[7] Advances in drug delivery systems, such as receptor-mediated transcytosis, lipophilic pathways, and paracellular pathways, have allowed the penetration of drugs through the BBB and, eventually, the treatment of IS.^[8–10] However, even if drugs cross the BBB, they may not directly reach the infarct area. Since the infarct area is directly related to blocked blood vessels, drugs administered through the bloodstream may not effectively reach the affected region. Alternatively, drug-carrying vehicles can be directly injected into brain tissues, and the release of the loaded drugs effectively treats IS. For example, drug-loaded

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RESEARCH ARTICLE

Morphological modulation enabled by non-halogenated solvent-processed simple solid additives for high-efficiency organic solar cells

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Abstract

The simple-structural and volatile solid additive 1,4-dibromobenzene (DBrB) can outperform organic solar cells (OSCs) fabricated with 1,4-diiodobenzene and 1,4-dichlorobenzene in terms of power conversion efficiency (PCE). A remarkable PCE of 17.0% has been achieved in a binary OSC based on DBrB-optimized photoactive materials processed from non-halogenated solvents, which is mainly attributed to the formation of a three-dimensional interpenetrating network and the orderly arrangement of the photoactive materials by improving the intermolecular interaction. This optimized morphology enables efficient charge transfer/transport as well as suppressed charge recombination, resulting in the simultaneous increase in all photovoltaic parameters. More importantly, we demonstrate that non-halogenated solvent-processed DBrB enabled PM6:Y6-HU OSCs with an impressive PCE of 18.6%, which is the highest efficiency yet reported for binary OSCs. This study suggests that the novel DBrB volatile solid additive is an effective approach to optimizing the morphology and thereby improves the photovoltaic performance of OSCs.

KEYWORDS

dibromobenzene, morphology optimization, non-halogenated solvent, organic solar cells, solid additives

Muhammad Haris, Du Hyeon Ryu, and Zakir Ullah equally contributed to this work.

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Article

Comparative Study of the Effect of Pollen Substitute Diets on Honey Bees during Early Spring

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Simple Summary: Recently, there has been a serious decline in honey bee colonies due to various factors, including habitat loss, diseases, pathogen infections, chemical exposures, and climatic changes. Among these factors, malnutrition-induced stress plays a significant role in colony decline. In our study, we present a novel perspective on maintaining and developing honey bee colonies. Our research demonstrates that a specific pollen substitute diet significantly improves colony performance during early spring, as evidenced by an increase in population, a greater capped brood area, altered colony weight, honey bee weight (dried head + thorax), and *vitellogenin* value. This finding could contribute to enhanced colony maintenance and development during the early spring season, with the potential to prevent colony decline.

Abstract: The nutritional quality of a colony significantly affects its health and strength, particularly because it is required for population growth in the early spring. We investigated the impact of various artificial pollen substitute diets on colony performance in the Republic of Korea during early spring, a critical period for colony health and growth. The colonies were provided with different diets, including the commercial product Megabee (positive control), our developed diet Test A, and four upgraded versions (Diet 1, Diet 2, Diet 3, and Diet 4) of Test A. The negative control group received no supplementary feed. Over 63 days, we observed 24 experimental colonies and assessed various parameters at the colony and individual levels. The results revealed that Diet 2 had the highest consumption and had the most positive impact on population growth, the capped brood area, colony weight, honey bees' weight, and *vitellogenin* levels. These findings suggested that Diet 2 is most attractive to honey bees and thus holds great promise for improving colony maintenance and development during the crucial early spring period.

Keywords: honey bee; substitute diet; consumption; brood area; population; colony weight; honey bees weight; *vitellogenin*; deformed wing virus



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1. Introduction

Honey bees (*Apis mellifera* L.) primarily rely on nectar and pollen as their sources of nutrition. They use nectar to produce honey, which serves as their primary carbohydrate source [1]. Carbohydrates are the primary component of a honey bee's diet and supply energy for various functions, including muscular activity, maintaining body heat, and supporting the essential operations of specific organs and glands, such as wax production [2]. Pollen provides a colony with nutritious proteins, lipids, vitamins, and minerals, all of which are required for the growth of broods [3]. Proteins have significant effects on physiological processes (immune response, parasite tolerance and survival, worker lifespan,

Article

Gut Microbiota of *Apis mellifera* at Selected Ontogenetic Stages and Their Immunogenic Potential during Summer

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Abstract: Honeybees (*Apis mellifera*) are pollinating agents of economic importance. The role of the gut microbiome in honeybee health has become increasingly evident due to its relationship with immune function, growth, and development. Although their dynamics at various developmental stages have been documented, their dynamics during the era of colony collapse disorder and immunogenic potential, which are connected to the antagonistic immune response against pathogens, need to be elucidated. Using 16S rRNA gene Illumina sequencing, the results indicated changes in the gut microbiota with the developmental stage. The bacterial diversity of fifth stage larva was significantly different among the other age groups, in which *Fructobacillus*, *Escherichia-Shigella*, *Bombella*, and *Tyzzzerella* were unique bacteria. In addition, the diversity of the worker bee microbiome was distinct from that of the younger microbiome. *Lactobacillus* and *Gilliamella* remained conserved throughout the developmental stages, while *Bifidobacterium* colonized only worker bees. Using an in silico approach, the production potential of lipopolysaccharide-endotoxin was predicted. Forager bees tend to have a higher abundance rate of Gram-negative bacteria. Our results revealed the evolutionary importance of some microbiome from the larval stage to the adult stage, providing insight into the potential dynamics of disease response and susceptibility. This finding provides a theoretical foundation for furthering the understanding of the function of the gut microbiota at various developmental stages related to probiotic development and immunogenic potential.

Keywords: honeybee; *Apis mellifera*; age; gut microbiome; *Lactobacillus*; *Gilliamella*; lipopolysaccharide



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1. Introduction

Apis mellifera (*A. mellifera*), otherwise known as social insects, are among the most important pollinators worldwide [1]. The health of *A. mellifera* colonies has been a major concern considering colony losses in recent decades due to colony collapse disorder (CCD) during various seasons including summer [2–4]. Thousands of hives appeared empty, with a massive disappearance of bees [5]. Various factors contribute to this incidence, including heavy use of pesticides, climate change, pathogen infection, and excessive abuse of antibiotics [6,7]. These factors affect honeybee behavior and feeding [8]. However, the honeybee gut microbiota is closely related to the host and provide the host with several advantages, including improving food digestion, pathogen defense, regulating honeybee behavior and immunity, and promoting development [9–11].

In arthropods such as honeybees, variation in the gut microbial climax community is linked to health and fitness instability [12]. Adult honeybees can harbor as many as 10⁹ bacterial cells; these cells constitute eight prevalent phylotypes that constitute up to 95% of the overall bacterial population [13]. Among these bacteria, *Lactobacillus* is usually the most abundant and widespread in the gut [14]. Nutrient status, such as the change from relying on pollen to nectar, social life, such as from nursing to foraging, and developmental



A single mutation in the mosquito (*Aedes aegypti*) olfactory receptor 8 causes loss of function to 1-octen-3-ol

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ABSTRACT

The host-seeking behavior of mosquitoes have long been established to be primarily odor-mediated. In this process, olfactory receptors (Ors) play a critical role. 1-Octen-3-ol is a common volatile compound that is attractive to hematophagous arthropods such as mosquitos. The olfactory receptor 8 (AaOr8) on the tip of the stylet and maxillary palp of *Aedes aegypti* is tuned to 1-octen-3-ol, which is required for mosquitoes to quickly find blood vessels from a vertebrate host. However, little is known about the interaction of AaOr8 with 1-octen-3-ol which was studied *in vivo* and *in silico* in this study. The molecular binding poses and energies between ligands and the receptor were investigated. Three mutants of AaOr8 were cloned and compared with *in vivo* calcium imaging utilizing heterologous expression systems. As a result, our findings imply that a genetic disruption including targeted modification of Ors genes may be used to reduce mosquito bites.

1. Introduction

Insect olfactory receptors are similar to hormone receptors and neuroreceptors in mammals and detect physiologically meaningful chemical ligands (Sokolinskaya et al., 2020). Affecting the responses of olfactory sensory neurons, they regulate a wide range of insect behavior, most importantly, those that can negatively impact human health (Zwiebel and Takken, 2004; Wang, 2012). For example, the mosquito *Aedes aegypti* transmits diseases including Zika, dengue, chikungunya, and yellow fever. Especially, female mosquitoes rely largely on the olfactory sense to find host animals for blood meals, which are required for egg production (Bowen, 1991; Kwon et al., 2006; Takken and Verhulst, 2013). Therefore, it is important to understand which of these sensory modalities may provide the most stable targets for the chemical disruption of human diseases, for designing effective vector control programs (Carrillo et al., 2023).

The mosquito's olfactory system detects volatile organic compounds (VOCs) released by the host in order to locate a suitable host. VOCs from animals are mainly detected by the binding of an odorant with the olfactory receptor (Or) embedded in the olfactory receptor neurons (Lu et al., 2007). Among the VOCs, 1-octen-3-ol (octenol) is a common odorant released by vertebrates that attract hematophagous arthropods such as mosquitoes when combined with carbon dioxide (CO₂) (Takken

and Kline, 1989; Grant et al., 1995; Grant and O'Connell, 2007; Xu et al., 2015). Octenol occurs naturally as two optically active enantiomers: (S)-(+ and R)-(–), with carbon-3 as the chiral center, and both of them attract of mosquitoes (Kline et al., 2007). In the case of the yellow fever mosquito, *Ae. aegypti*, Bohbot and Dickens (2009) reported a finding: olfactory receptor 8 (AaOr8) acts as a chiral selective receptor specifically for the (R)-(–)-enantiomer of 1-octen-3-ol. Later, Jung et al., 2015 have shown that the AaOr8 of the mosquito *Ae. aegypti* is located on the stylet and in the maxillary palps, in conjunction with the olfactory co-receptor (AaOrco), and selectively responds to octenol in a time and concentration-dependent manner. Specifically, Or8 mRNA modulation was found octenol exposure dependent (Mappin et al., 2023). AaOr8 expressed in maxillary palps, detects VOCs from a distance, while AaOr8 in stylet, detects VOCs at short distances and blood vessels under the skin, allowing blood to be sucked (Bohbot et al., 2013; Jung et al., 2015). However, the Or8 was found in the non-blood-sucking mosquito genus *Toxorhynchites* and it also detects octenol that exists in nature (Dekel et al., 2016). This suggests that Or8 is a highly conserved receptor in mosquitoes (Robertson et al., 2003; Zhou et al., 2012). However, the molecular mechanisms that determine AaOr8 ligand selectivity are poorly understood yet compared with mammalian Or's.

The first molecular structure of the Ors was elucidated in mammals and is known as a 7-transmembrane G protein-coupled receptor (GPCR)

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Article

Monitoring Immune Modulation in Season Population: Identifying Effects and Markers Related to *Apis mellifera ligustica* Honey Bee Health

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Abstract: Honey bees play a significant role in ecology, producing biologically active substances used to promote human health. However, unlike humans, the molecular markers indicating honey bee health remain unknown. Unfortunately, numerous reports of honey bee collapse have been documented. To identify health markers, we analyzed ten defense system genes in *Apis mellifera ligustica* honey bees from winter (Owb) and spring (Fb for foragers and Nb for newly emerged) populations sampled in February and late April 2023, respectively. We focused on colonies free from SBV and DWV viruses. Molecular profiling revealed five molecular markers of honey bee health. Of these, two seasonal molecular markers—*domeless* and *spz* genes—were significantly downregulated in Owb compared to Nb and Fb honey bees. One task-related marker gene, *apid-1*, was identified as being downregulated in Owb and Nb compared to Fb honey bees. Two recommended general health markers, *SOD* and *defensin-2*, were upregulated in honey bees. These markers require further testing across various honey bee subspecies in different climatic regions. They can diagnose bee health without colony intervention, especially during low-temperature months like winter. Beekeepers can use this information to make timely adjustments to nutrients or heating to prevent seasonal losses.

Keywords: honey bee; health markers; innate immunity; defense system; antioxidant system; ROS enzymes; *Apis mellifera ligustica*



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1. Introduction

Honey bees play a significant role in ecology and produce biologically active substances that humans have long used to promote health. Various aspects of health, including at the molecular, microbial, and population levels, have recently gained increased interest from researchers, stakeholders, and citizens [1]. However, unlike humans, the standard parameters of honey bee health remain unclear. To investigate this, it is crucial to consider the different castes and ages of honey bees present in a colony, including the queen, drone, nurses, and forager workers [2,3], as well as the two types of workers: the long-lived winter population and the short-lived summer population [4]. While short-lived honey bees live for about 35–45 days, long-lived honey bees can survive up to six months [5]. Short-lived workers perform different tasks in an age-dependent sequence: newly emerged bees transform into young workers (nurses), which usually care for the brood during the first two or three weeks of adult life, while older workers (foragers) forage for nectar and pollen outside the nest [6]. In contrast to short-lived bees, long-lived honey bees form winter clusters, enabling colony thermoregulation during the 3–4 months of winter. After

A new species of *Siccia* (Lepidoptera, Erebidae, Arctiinae) from Vietnam

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Abstract

A new species *Siccia triellipsis* sp. nov., is described from Vietnam. Illustrations of the adults and genitalia of the new species are provided.

Key words: Lithosiini, distribution, Oriental region, taxonomy

Introduction

Siccia Walker (1854) is one of the large genera within the tribe Lithosiini (Erebidae, Arctiinae), which is widely distributed in the Old World. It was established for its type species, *Siccia caffra* Walker, 1854 found in South Africa (Cape Colony, Knysna). Recently, Volynkin (2023a) provided a checklist of Asiatic species of this genus while studying Afrotropical species. Members of this genus are small to middle-sized moths with a pale, grayish ground color on the forewing, featuring a pattern of dark spots and transverse lines, or reduced to a few and often just one spot (typically the discal one). They have very diverse male and female genital structures (Volynkin 2023a).

Asian *Siccia* was studied by several authors such as Fang (2000), Černý and Pinratana (2009), Kishida (2010, 2011), Bucsek (2012, 2014), Bayarsaikhan *et al.* (2017), Volynkin (2023b), Volynkin & Černý (2023), etc.

In Vietnam, 10 species of *Siccia* have been reported by Volynkin (2023a, b): *S. annamica* (Dubatolov & Bucsek, 2016), *S. monastyrskii* (Dubatolov & Bucsek, 2013), *S. punctata* Fang, 2000, *S. sordida* (Butler, 1877), *S. angusta* Volynkin, 2023, *S. gryllotalpa* Volynkin, 2023, *S. dilatata* Volynkin, 2023, *S. suffusa* Volynkin, 2023, *S. spinifera* Volynkin, 2023, and *S. megala* Volynkin, 2023 (Volynkin 2023a, b). The propose of this study is to describe a new *Siccia* species from BaVi National Park, Hanoi Prov. (Hatay), Vietnam (Fig. 1).

Materials and Methods

Specimens used in this study were collected during expeditions to Vietnam (2005–2014), mostly in four national parks: Ba Be National Park, Tam Dao National Park, BaVi National Park, and Cuc Phuong National Park. The specimens were collected using light traps with a mercury vapor lamp (220V/200W, Dongseong Co., Korea) and four black light lamps (FL20SbL, Kumho Co., Korea). Genitalia were dissected and examined under Leica EZ4 stereo-microscope. Images of adults and genitalia were taken using a Cannon EOS 50D digital camera attached to a Cannon EF 100 mm F2.8 Macro USM lens (Cannon, Inc., Tokyo, Japan) and a Leica DM 2500 microscope attached with Leica ICC50 E (Leica, Wetzlar, Germany). All examined species of Vietnam are deposited in Incheon National

Hydrogen-Bonding Interactions Between Terpolymers Enable Excellent Device Efficiency and Operational Stability of Non-Halogenated Solvent-Processed Polymer Solar Cells

Fiza Arshad, Muhammad Haris, Eun Sung Oh, Zakir Ullah, Du Hyeon Ryu, Seungjin Lee, Hang Ken Lee, Sang Kyu Lee, Taek-Soo Kim,* Hyung-Wook Kwon,* Chang Eun Song,* and Won Suk Shin*

Although polymer solar cells (PSCs) have shown considerable power conversion efficiency (PCE) potential, their poor operational stability is a major obstacle for their future commercialization. In this study, the ternary-blend strategy based on D1–A–D2-type conjugated random terpolymers containing hydrogen-bonding sites is employed to simultaneously improve device efficiency and long-term stability. Notably, the PM6-ThEG:PM6-ThOH:Y6-BO ternary-blend system exhibits a remarkable PCE of 17.2% with superior photo, thermal, and mechanical stability, outperforming those of binary devices based on PM6, PM6-ThEG, and PM6-ThOH polymer donors. These outstanding results are likely attributed to the robust molecular lock via hydrogen bonds between PM6-ThEG and PM6-ThOH terpolymers, which can induce strong intermolecular packing, a dense 3D terpolymer network, and optimized morphology. These results also correlate well with the computational study. A comprehensive analysis of optoelectronic and morphological properties as well as the exploration of underlying physical mechanisms collectively verifies the effectiveness of this approach based on mixed random terpolymers with hydrogen-bonding moiety to achieve the non-halogenated solvent-processed PSCs with exceptional efficiency and operational stability.

1. Introduction

Polymer solar cells (PSCs) have gained much attention as renewable photovoltaic technology because of their transparency, light weight, and low-cost solution processing compatible with roll-to-roll manufacturing.^[1–3] Over the past two decades, tremendous progress has been made in the field of PSCs to boost the power conversion efficiency (PCE) from 7% to 19% by concerted efforts of material development and device engineering.^[4–12] However, there has been less focus on both operational stability and eco-friendly processing of PSCs, despite it being crucial to meet these requirements for practical outdoor applications.^[13]

PSC instability originates mainly from two degradation models.^[14] First, interfaces at the organic photoactive film/charge-transport layer or electrode are likely to undergo chemical degradation, particularly

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Article

The Effects of Artificial Diets on the Expression of Molecular Marker Genes Related to Honey Bee Health

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Abstract: Honey bees are commonly used to study metabolic processes, yet the molecular mechanisms underlying nutrient transformation, particularly proteins and their effects on development, health, and diseases, still evoke varying opinions among researchers. To address this gap, we investigated the digestibility and transformation of water-soluble proteins from four artificial diets in long-lived honey bee populations (*Apis mellifera ligustica*), alongside their impact on metabolism and DWV relative expression ratio, using transcriptomic and protein quantification methods. Diet 2, characterized by its high protein content and digestibility, was selected for further analysis from the other studied diets. Subsequently, machine learning was employed to identify six diet-related molecular markers: *SOD1*, *Trxr1*, *defensin2*, *JHAMT*, *TOR1*, and *vg*. The expression levels of these markers were found to resemble those of honey bees who were fed with Diet 2 and bee bread, renowned as the best natural food. Notably, honey bees exhibiting chalkbrood symptoms (Control-N) responded differently to the diet, underscoring the unique nutritional effects on health-deficient bees. Additionally, we proposed a molecular model to elucidate the transition of long-lived honey bees from diapause to development, induced by nutrition. These findings carry implications for nutritional research and beekeeping, underscoring the vital role of honey bees in agriculture.

Keywords: *Apis mellifera*; insect model; nutrition; water-soluble proteins; innate immunity; winter honey bees; early spring; bee bread; functional food



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1. Introduction

Honey bees produce many products for human health and play a crucial ecological role in both natural ecosystems and agriculture. Similar to other insects, honey bees present both an alternative source of protein for humans and a convenient animal model for fields from genetics to neurobiology, especially for the early stage preclinical research of pathogen infection experiments, innate immune system research, and nutrition effects [1,2].

These benefits of honey bees are available because insects and humans possess some equivalent organs and similar biological systems, including the evolutionarily conserved gut and digestive system [1]. Malnutrition, overfeeding, high-sugar diet (HSD), and high-fat diet (HFD) effects on metabolism in a *Drosophila* model were described [3–5], with a focus on their effects on lifespan, health, age-related diseases, and aging [6–8]. Next, nutrition, proteins in the diet, and their transformation from food to organism are critical, as animals construct their bodies from amino acids derived from animal and plant foods, which are subsequently passed through the food chain. For instance, proteins are responsible for the development of the fat body in the abdomen of individual honey bees, influencing energy storage, production, and the secretion of vitellogenin (vg) protein, antimicrobial peptides, and components of the humoral immune response [9]. Despite the obvious importance of proteins for animals, our understanding of their effects on health and development still



Establishment of a new promoter trapping vector using 2A peptide

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Abstract

Promoter trapping is a powerful tool for discovering promoters and uses promoter trapping vectors. However, the traditional trapping vector allows expression even if it does not integrate into the host cell genome, and even if it does integrate into the genome, it is more likely to integrate in a region other than the promoter region. In this study, to overcome the shortcomings of traditional trapping vectors, we used the bicistronic 2A system to link GFP and the neomycin resistance gene. Because this vector does not contain a promoter, simultaneous production of GFP and neomycin resistance protein requires integration into the promoter region. In fact, GFP expression was observed in more than 90% of the cell clones that survived in the medium containing antibiotics, confirming that the 2A system operates. The vector insertion location was confirmed through whole genome sequence analysis, and a 1-kb promoter candidate region was selected through promoter motif analysis. In fact, a 1-kb region inserted into a promoterless luciferase expression vector showed strong promoter activity, demonstrating its utility as a tool to find promoters. In summary, we constructed a novel promoter trapping vector using the 2A system and used it to discover the promoter with strong activity. This vector will increase the efficiency of promoter trapping, providing an opportunity to easily discover new promoters in mammalian cells.

Keywords Promoter trapping · Bicistronic 2A system · Trapping vector

1 Introduction

Improving the protein productivity in mammalian cells has been a major research goal in the biopharmaceutical field, and approaches have been attempted from three aspects: process, cell line, and expression vector [1]. However, there are limits to controlling protein productivity simply by improving the process such as pH, media, dissolved oxygen concentration, and stirring speed [2–4]. Moreover, improvement of cell lines through gene editing is progressing slowly

because it is not well known which genes play an important role in protein productivity [5, 6]. However, improvements in expression vectors have been more effective in increasing protein productivity than other methods by increasing transcription efficiency [7, 8]. Therefore, there is increasing research interest in improving expression vectors.

The expression vector consists of a coding sequence (CDS), a promoter driving CDS expression, and a transcription terminator for the CDS [7]. In expression vectors, gene expression is controlled by promoters, and protein production efficiency is closely related to the strength of promoter activity [9, 10]. Therefore, the discovery and characterization of promoters with higher activity is important for protein production [11]. Commonly used promoters for protein production are the simian virus 40 (SV40) or cytomegalovirus (CMV) promoters [12]. These virus-derived promoters enable the expression of large amounts of recombinant proteins, but tend to be epigenetically silenced, ultimately reducing protein productivity [13, 14]. These shortcomings have been largely compensated for by using promoters found in Chinese hamster ovary (CHO) cells. One successful example is the promoter of the Chinese hamster elongation

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Intestinal microbial diversity in domestic honey bees and evaluation of their probiotic properties

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ABSTRACT

As populations of managed honey bees in various regions increase in comparison to wild colonies, there is a need to ensure that threats to bee health and survival in all environments are countered. One of the more direct methods of approaching bee health is through dietary means and as such, we investigated the intestinal microbial diversity in the gut of honey bees in order to identify strains with probiotic potential. Honey bees were fed with several feeds including Feedbee, Megabee, sugar syrup 60% (w/v), Yangbongnonghyup (YBNH), and Test A, to monitor the culture-dependent and culture-independent microbial diversity. In the culture-dependent study, most isolates belonged to *Apilactobacillus* (24.1%), *Fructobacillus* (21.8%), and *Bacillus* (15.4%) at the genus level. Culture-independent results showed that *Bacillota* and *Pseudomonadota* were the most abundant groups (99%) among the gut bacteria, while other bacteria showed a lower percentage than 0.1%. Next, the hydrolytic enzymes (α -amylase, protease, and cellulase) and antibacterial and antioxidant activities of the isolated strains were evaluated to examine their applicability as potential probiotics for honey bees. The selected strains showing three hydrolytic enzyme activities were used to determine their antibacterial activity against *Paenibacillus larvae*. *Bacillus subtilis* MBLB2796 showed the highest α -amylase (1.24 ± 0.06 U/mL) and cellulase (0.24 ± 0.01 U/mL) activities while *B. tequilensis* MBLB2834 exhibited the highest protease activity (0.15 ± 0.03 mU/mL) and the highest antioxidant activity ($71.0 \pm 3.8\%$). This study suggests that honey bee gut-originated isolates are prime candidates for use as probiotics in honey bees.

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Microbial diversity; honey bee; gut microbiome; *Bacillus subtilis*

Introduction

Honey bees (*Apis mellifera* L.) are globally recognized as highly valued insects, not only economically but also ecologically, as they are important pollinating insects and honey producers (Batra, 1995; Dedej & Delaplane, 2003; Vanengelsdorp & Meixner, 2010). The health of honey bees is increasingly threatened by climate change, destroyed habitats, pesticides, parasites and pathogens. Therefore, the health of honey bees has become a major concern in many countries (Goulson et al., 2015).

The microbiota in the guts of animals can benefit their hosts and play a critical role in their health. Microorganisms in the honey bee gut colonize their digestive tracts and can affect their hosts by helping to metabolize dietary carbohydrates, detoxify harmful molecules, provide essential nutrients, contribute to resistance to diseases and parasites, and modulate

development and immunity (Engel & Moran, 2013). The insect gut is considered an organ with high microbiological diversity, where pathogen growth, including viruses, fungi, bacteria, and protozoa is efficiently suppressed by probiotic microbiota (Elzeini et al., 2021). One of the most critical honey bee diseases is American foulbrood (AFB), caused by the larvae of the Gram-positive spore-forming bacterium *Paenibacillus larvae* (Genersch et al., 2006). AFB is contagious, and an infection that begins in an individual honey bee larva can disrupt the entire colony (Ansari et al., 2017). To explore alternative and proficient control measures for AFB, Evans and Armstrong (2006) presented a novel method to inhibit *P. larvae* using microorganisms isolated from the honey bee gut. Numerous studies have shown that the consistency of the occurrence of specific microbial groups in bees in the hive provides clues to key gut microbiota and microbial types likely to

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Article

Enhancing Honey Bee Health: Evaluating Pollen Substitute Diets in Field and Cage Experiments

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Simple Summary: Globally, honey bee colonies face numerous threats, such as habitat loss, diseases, chemical exposure, and climate change, with malnutrition being a significant factor in colony decline. Beekeepers struggle to provide adequate pollen during seasonal pollen dearth when it is not readily available to honey bees, prompting the use of pollen substitute diets to support colonies. Our study compared a new pollen substitute diet with a control through field and cage experiments, finding that Diet 1 significantly improved honey bee health. This was evidenced by an increase in population, a greater brood area, higher consumption levels, preference, altered colony weight, honey production, honey bee weight (dried head and thorax), and *vitellogenin* expression levels. These improvements were correlated with the amino acid content of each diet, offering potential solutions to boost honey bee health and combat colony decline.

Abstract: Honey bees (*Apis mellifera* L.) play vital roles as agricultural pollinators and honey producers. However, global colony losses are increasing due to multiple stressors, including malnutrition. Our study evaluated the effects of four pollen substitute diets (Diet 1, Diet 2, Diet 3, and Control) through field and cage experiments, analyzing 11 parameters and 21 amino acids. Notably, Diet 1 demonstrated significantly superior performance in the field experiment, including the number of honey bees, brood area, consumption, preference, colony weight, and honey production. In the cage experiment, Diet 1 also showed superior performance in dried head and thorax weight and *vitellogenin* (*vg*) gene expression levels. Canonical discriminant and principle component analyses highlighted Diet 1's distinctiveness, with histidine, diet digestibility, consumption, *vg* gene expression levels, and isoleucine identified as key factors. Arginine showed significant correlations with a wide range of parameters, including the number of honey bees, brood area, and consumption, with Diet 1 exhibiting higher levels. Diet 1, containing apple juice, soytide, and *Chlorella* as additive components, outperformed the other diets, suggesting an enhanced formulation for pollen substitute diets. These findings hold promise for the development of more effective diets, potentially contributing to honey bee health.

Keywords: honey bee; pollen substitute diets; nutrition; health; development; amino acid contents



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1. Introduction

Honey bees (*Apis mellifera* L.) are vital agricultural pollinators cultivated for honey and other hive products [1,2]. Globally, high honey bee colony losses occur due to the effects of multiple stressors [3]. This decline in honey bee populations has had adverse impacts on both the economy and ecology, posing a significant threat to crop production and human well-being [4,5]. Several factors contribute to the decline, including habitat loss, diseases, pathogen infections, chemical exposures, and climatic changes [3,6,7]. Among these factors, malnutritional stress plays a significant role in colony decline [6]. The quality of a colony's



Original Article

An annotated checklist of the Arctiinae of Korea (Lepidoptera: Noctuoidea, Erebidae) with two newly recorded genera

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ABSTRACT

An annotated checklist of the Korean Arctiinae (Noctuoidea: Erebidae) is presented, containing 76 species in 40 genera. Two genera of Lithosiini, *Philenora* Rosenstock, 1885, for the species *P. latifasciata* Inoue & Kobayashi, 1963, from Goeje Is., and *Ammatho* Walker, 1855, for *A. cuneonotatus* Walker, 1855, from Ulleung Is., are recorded for the first time in Korea. Illustrations of adult and genitalia figures of the newly recorded species are provided with an updated checklist.

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Introduction

Korean arctiine moths have been studied by a substantial number of researchers. The first record was by Butler (1882), who reported one species, *Stigmatophora micans* (Bremer & Grey, 1852). Since then, foreign and native workers who have contributed to the knowledge of Korean Arctiinae moths include Fixsen (1887), Leech (1888), Alphéraky (1897), Hampson (1900, 1901), Seitz (1912), Okamoto (1924), Matsumura (1927, 1931), Maruta (1929a, 1929b), Kamijo (1933), Doi (1939), Bryk (1948), Inoue (1961), Pak (1964), Kim et al. (1967, 1982), Choi (1968, 2004, 2010), Lee (1975), Nam et al. (1980), Shin and Han (1982), Nam et al. (1985), Park (1976, 2000), Kim (1999), Park et al. (2001), Choi (2010), Bae et al. (2013), Lee et al. (2015), Choi and Kim (2016), Przybyłowicz et al. (2016),

Bayarsaikhan et al. (2016a, 2016b, 2017, 2020, 2022a, 2022b), and Choi et al. (2020, 2021).

The Korean Arctiinae fauna has been cataloged by Park (2000), Bae et al. (2013), and Bayarsaikhan et al. (2017). These comprehensive works include information on species, synonyms, descriptions, distributions, host plants, and the earliest recorded dates in Korea, as well as illustrations of the adults and genitalia. Also, there are many articles published containing only new species, newly recorded species descriptions, or generic reviews. The most recent study of Arctiinae in Korea was provided by Bayarsaikhan et al. (2017), with an illustrated catalog of 71 species in 39 genera belonging to three tribes.

In the present study, the checklist of the Arctiinae of Korea is revised and incorporates a number of corrections and changes to recent classifications and nomenclature as presented in the most recent checklist. Two genera, *Rhyparia* Hübner, [1820] 1816, and *Rhyparioides* Butler, 1877, have been synonymized with *Diacrisia* Hübner, [1819] 1816, by Rönkä et al. (2016), based on the results of a phylogenetic study. Following these results, five Korean species, *Rhyparia purpurata* (Linnaeus, 1758), *Rhyparioides amurensis* (Bremer, 1861), *R. metelkana* (Lederer, 1861), *R. nebulosa* Butler,

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Review of the genus *Diduga* (Lepidoptera, Erebidae, Arctiinae) of Vietnam, with a new species and two newly recorded species

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Abstract

Species of the genus *Diduga* Moore are reviewed in Vietnam, with nine species including a new species, *D. laocai* sp. nov., and two newly recorded species, *D. hastata* Bayarsaikhan & Bae, 2021 and *D. robdevosi* Bayarsaikhan & Bae, 2023. Illustrations of adults and male and female genitalia of all examined species are provided.

Key words: taxonomy, Lithosiini, checklist, Oriental Region

Introduction

One of the diverse genera, *Diduga* Moore of the tribe Lithosiini (Erebidae, Arctiinae) was erected for its type species, *Diduga costata* Moore, [1887], from Sri Lanka. To date, this genus comprises 48 species, widely distributed in the Oriental Region (Bayarsaikhan *et al.* 2023a, 2023b). During the examination of extensive Lithosiini materials deposited in the McGuire Center for Lepidoptera and Biodiversity (Florida) we found a more undescribed species from Vietnam.

In Vietnam, six species of *Diduga* have been reported by Dubatolov & Bucsek (2016), Bayarsaikhan & Bae (2019), and Bayarsaikhan *et al.* (2023a). In this study, we describe an additional new species and two newly recorded species from Vietnam. Nine species of *Diduga* are currently known from Vietnam.

Materials and methods

Specimens examined in this study are deposited in the McGuire Center for Lepidoptera and Biodiversity (MGCL), Gainesville, USA, and the Incheon National University (INU), Incheon, Republic of Korea. Abbreviations used are as follows: TS = type species; TL = type locality.

Article

Identification of Cellular Isoschaftoside-Mediated Anti-Senescence Mechanism in *RAC2* and *LINC00294*

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Abstract: As cellular senescence, reactive oxygen species (ROS) accumulate excessively, causing cellular damage. Flavonoids derived from natural products are known for their antioxidant effects and their ability to delay cellular senescence. Previous studies have attempted to mitigate cellular senescence using flavonoids from natural sources. However, the detailed mechanisms and regulatory targets of some flavonoids exhibiting antioxidant effects have not been fully elucidated. Therefore, we screened a library of flavonoids for antioxidant properties. Isoschaftoside, a glycosidic flavonoid, significantly reduced ROS levels in senescent cells. It was found that mitochondrial function was restored, and dependence on glycolysis was reduced in senescent cells treated with isoschaftoside. Additionally, we identified that isoschaftoside suppresses ROS by reducing the expression of *RAC2* and *LINC00294* in senescent cells. Taken together, this study establishes a novel mechanism for ROS inhibition and the regulation of cellular senescence by isoschaftoside. Our findings contribute important insights to antioxidant and anti-senescence research.

Keywords: senescence amelioration; flavonoid; isoschaftoside; reactive oxygen species (ROS)



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1. Introduction

The skin consists of three layers (the epidermis, dermis, and subcutaneous fat) and is an epithelial tissue that covers our body [1]. It acts as a barrier to the environment, protects against microorganisms, and maintains body fluids and temperature [1]. Skin aging is the process by which the quality of the cells that make up skin tissue deteriorates with age. This process leads to the thinning of the epidermis and the flattening of the dermal–epidermal junction [2]. As cells age, epidermal cell turnover decreases, resulting in poorer exfoliation and slower wound healing [2]. The dermis undergoes the most significant microstructural changes during aging. Deterioration in the dermis involves the separation of the dermis and epidermis, leading to looser skin and a reduced regeneration of epidermal stem cells [2]. Fibroblasts, the most common cells in the dermis, decrease in both number and function with age [2]. Skin aging changes the function of cellular organelles, especially with regard to mitochondrial degeneration [3]. As skin ages, defective mitochondria accumulate and undergo structural changes that significantly increase mitochondrial volume and size [3]. Strategies are needed to restore the functionality of structurally and functionally aged skin.



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Review Article

Snouted tiger moth (Noctuoidea, Erebiidae, Aganainae) in Korea with two newly records of *Asota*: A connection with LepidopterismUlzijargal Bayarsaikhan^{a,b,c}, Jae-Ho Ko^d, Yeong-Don Lee^e, Ki-Seok Oh^f,
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ABSTRACT

The subfamily Aganainae is represented in Korea by seven species in two genera, *Mecodina subviolacea* (Butler), *M. cineracea* (Butler), *M. nubiferalis* (Leech), *M. subcostalis* (Walker), *Asota egens* (Walker), *A. caricae* (Fabricius), and *A. plaginota* (Cramer). Among these, *A. caricae* (Fabricius) and *A. plaginota* (Cramer) are new records to the moth fauna of Korea. Illustrations of adults and genitalia of all examined species are provided.

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Introduction

The subfamily Aganainae belonging to the family Erebiidae, includes 109 described species in 11 genera worldwide (Fibiger and Lafontaine 2005; Zahiri et al. 2012), excluding the genus *Mecodina* Guenée, 1852. Species of this subfamily are mostly large, robust, colorful moths, both as larvae and adults. They are often aposematic day flyers and are widely distributed in the tropical zone (Barlow 1982; Holloway 1988; Kononenko and Pinratana 2013; Bayarsaikhan et al. 2016; Spitsyn and Bolotov 2020).

First record of Korean Aganainae (Aganainae misspl.) was listed by Kim et al. (2016) with four species in *Mecodina*, *M. subviolacea* (Butler 1881), *M. cineracea* (Butler 1879), *M. nubiferalis* (Leech 1889), and *M. subcostalis* (Walker 1865), based on recent study

of Zahiri et al. (2012) and Kononenko and Pinratana (2013). Lee et al. (2021) reported the second genus *Asota* Hübner for one species, *A. egens* (Walker 1854) from Gangwon Province, Korea.

The purpose of this paper is to study the subfamily Aganainae in Korea, and present two newly recorded species, *A. caricae* (Fabricius) and *A. plaginota* (Cramer). The placement of *Mecodina* in the family Erebiidae and subfamily Aganainae in the present study is as per Zahiri et al. (2012) and Kononenko and Pinratana (2013). Illustrations of adults and genitalia of all examined species are provided.

Material and methods

The specimens used in this study are deposited in the collections of Incheon National University (INU), Incheon, Republic of Korea, Honam National Institute Biological Resources (HNIBRIN), Mokpo, Republic of Korea, and Natural History Museum, Kyung Hee University (NHM), Seoul, Republic of Korea. Images of adults were taken by using a Cannon EOS 50D digital camera attached to a Cannon EF 100 mm F2.8 Macro USM lens (Cannon, Inc., Tokyo,

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Article

The Improving Effects of Probiotic-Added Pollen Substitute Diets on the Gut Microbiota and Individual Health of Honey Bee (*Apis mellifera* L.)

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Abstract: Honey bee (*Apis mellifera* L.) health is crucial for honey bee products and effective pollination, and it is closely associated with gut bacteria. Various factors such as reduced habitat, temperature, disease, and diet affect the health of honey bees by disturbing the homeostasis of the gut microbiota. In this study, high-throughput 16S rRNA gene sequencing was used to analyze the gut microbiota of honey bees subjected to seven diets over 5 days. *Lactobacillus* dominated the microbiota in all diets. Cage experiments (consumption, head protein content, and *vitellogenin* gene expression level) were conducted to verify the effect of the diet. Through a heatmap, the Diet2 (probiotic-supplemented) group was clustered together with the Beebread and honey group, showing high consumption (177.50 ± 26.16 mg/bee), moderately higher survival duration (29.00 ± 2.83 days), protein content in the head (312.62 ± 28.71 µg/mL), and diet digestibility ($48.41 \pm 1.90\%$). Additionally, we analyzed the correlation between gut microbiota and health-related indicators in honey bees fed each diet. Based on the overall results, we identified that probiotic-supplemented diets increased gut microbiota diversity and positively affected the overall health of individual honey bees.

Keywords: *Apis mellifera* L.; probiotics; gut microbiome; honey bee health; pollen substitute diet

1. Introduction

Honey bees (*Apis mellifera* L.), with their remarkable pollination abilities, are important contributors to promoting agricultural development and maintaining the ecological balance of nature. However, widespread honey bee colony losses are observed globally due to parasitic infections as well as various stressors [1]. Pathogen infections, pesticide exposure, and nutritional stress are major contributors [2]. Honey bees suffer from low nutrition and can transition to preforagers from nurse bees, reducing their survival [3].

Adequate colony nutrition, comprising sufficient protein and carbohydrate reserves, is critical for honey bees to withstand the challenges of modern apiculture [4]. Pollen, the primary source of protein and lipids, and nectar or honeydew, which provide carbohydrates, are essential components of honey bee nutrition [4]. The estimated daily pollen consumption of an individual adult honey bee ranges from 3.4 to 4.3 mg [5]. Consequently, the annual pollen requirement for an entire colony is estimated to be between 13.4 and 17.8 kg [5]. The term “beebread” refers to the pollen collected by honey bees, which is then processed, fermented, and stored in the hive to provide food for the nurse bees [6].



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Full length article

Dissemination of phosphorescent microbial agents by honey bees (*Apis mellifera* L.) in apple orchardsJin Hyeok Choi^a, Soon-Il Kim^b, Woohyung Lee^c, Yong Hwan Lee^c, Kil Won Kim^a,
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Phosphorescence

ABSTRACT

Honey bees are commonly used for pollination in apple orchards. This study aimed to develop a bee-vectoring scheme whereby honey bees (*Apis mellifera* L.) deliver microbial agents (*Bacillus amyloliquefaciens* MBI600) against fire blight from a dispenser at the entrance of the beehive to apple flowers. The efficiency of disseminating phosphorescent microbial agents (PMA) by honey bees was compared across four apple orchards; (A) with installed insect nets, (B) isolated on an island, (C) adjacent to other apple orchards and apiaries, and (D) near C within 50 m and without beehives. The average detection ratio of PMA on bees visiting flowers was 100 % in orchard (A) and (B), whereas in (C) and (D) it was 54.5 % and 40.0 %, respectively. Detection of PMA on apple blossoms was also 100 % in (A) and (B), but significantly low in (C) and (D) at 52.8 % and 12.9 %. In orchards (A) and (B), PMA adhered to pistils at high ratios of 98.0 % and 96.3 %, while in orchard (C), it was significantly lower at 9.3 %. Phosphorescence intensity in flowers and the proportion of apple pollen loads collected from incoming bees were also investigated. Finally compared to the orchard (A) and (B), open orchard (C) and (D) showed significantly lower microbial agent spread efficiency by honey bee vectoring. For open orchards, supplemental strategy to enhance bee vectoring could include increasing the number of hives in the orchard and temporarily relocating neighbor's beehives during flowering, or supplying additional dispensers to nearby apiaries.

Introduction

Bee colonies placed in an orchard for pollination during bloom periods allow many bees to act as highly effective and labor-saving vectors for applying biological control agents compared to direct spraying devices. Attaching dispensers to beehive entrances let bees to load and disseminate control agents during their foraging activities. Various bee pollinators have been studied in the development of this 'bee-vectoring' technology for biological control (Peng et al., 1992; Maccagnani et al., 2006; Kevan et al., 2008; Mommaerts and Smagghe, 2011).

Honey bees and bumble bees are commercially available year-round, even in the winter season. In contrast, solitary *Osmia* bees are restricted to the specific season. Bumble bees forming smaller colonies with a few

dozen workers are preferred for small-scale pollination tasks such as tomatoes and sweet peppers in green houses (Dimou et al., 2008; Yoon et al., 2021). The dispensers for *Osmia* and *Bombus* bees have developed for transferring microbiological control agents (Maccagnani et al., 2006; Mommaerts et al., 2010; Park et al., 2013). Various dispensers of microbial agents for honey bees also have been designed and tested (Peng et al., 1992; Thomson et al., 1992; Sutton and Peng, 1993).

The causative bacterium of fire blight disease in apple and pear, *Erwinia amylovora*, was designated as a prohibited quarantine pathogen in South Korea. Fire blight was the first reported in 2015, with a slight decrease observed until 2018 and rapid increase from 2019 onwards (Ham et al., 2020). In the event of fire blight occurrence, the government conducts initial diagnosis, followed by the excavation and burial of host

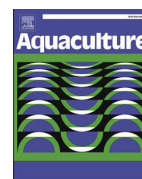
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Review

The Chinese mitten crab (*Eriocheir sinensis*) and its microbiome: A review

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ABSTRACT

The Chinese mitten crab also known as *Eriocheir sinensis*, is an economically valuable species widely farmed in China and South-East Asia. Belonging to the subphylum Crustacea, *E. sinensis* are good examples of catadromous species that inhabit freshwater environment but migrate to saltwater for spawning. These crabs host diverse microbial communities across all major organs including gills, hepatopancreas, gut, and hemolymph. Migration, driven by environmental changes and stress significantly influence the diversity, community compositions, and function of the crab microbiome. These symbiotic microbes play crucial roles in crab health, nutrition, taste and diseases. Despite significant research on the intestinal microbiota of *E. sinensis*, the microbiome associated with other organs remains unexplored. This review, aims to consolidate current knowledge on the *E. sinensis* microbiome, examining the dynamics, functional roles in the host, and the impact of stress on the microbiota composition. Additionally, we identify knowledge gaps and explore possible solutions to enhance and preserve beneficial microorganisms, essential for optimizing aquaculture in the host practices. This review is the first comprehensive analysis of the microbiome associated with *E. sinensis* across various physiological states, providing critical insights for the sustainable management of this aquaculture species.

1. Introduction

The microbiota plays a crucial role in host health and metabolism, including regulating immunity, antagonizing pathogenic bacteria, and participating in nutrient absorption and vitamin synthesis (Bikel et al., 2015). Microbiotas are integral to various host body parts, including the intestine, hemolymph, and hepatopancreas (Fig. 1 and Table 1). The animal intestine is a complex ecosystem of microorganisms that enhance digestive efficiency, nutrient utilization, immune system function, and inhibit the adhesion of opportunistic pathogens (Rungrassamee et al., 2014; Verner-Jeffreys et al., 2003; Gallo and Nakatsuji, 2011). In crustaceans, the intestinal microbiota is vital for maintaining ecological and physiological functions (Zhang et al., 2020a, 2020b) and sustaining homeostasis and immunity (Huang et al., 2018; Qi et al., 2022).

Environmental changes can lead to variability in the gut microbiome, affecting development, immunity, nutrition, and disease resistance (Sun et al., 2018). Multiple studies have shown that pollutants can disrupt the intestinal microbiota of aquatic organisms (Pelusio et al., 2020; Qiao et al., 2019), with stressors like ammonia and thermal variations altering bacterial community structures (Duan et al., 2021). For instance, imidacloprid exposure reduces beneficial bacteria and increases pathogens in the gut (Hong et al., 2020).

The hepatopancreas, critical for digestion and nutrient absorption, also plays a key role in innate immunity in invertebrates and hosts pathogens (Ceccaldi, 1989; Leano et al., 1998; Jiang et al., 2014). Hemolymph is essential in the host immune process, and healthy crustaceans' hemolymph hosts unique microbiota (Potgieter et al., 2015; Wang and Wang, 2015). However, there is limited knowledge about the

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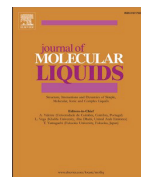
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Elucidating the interactions of advanced glycation end products with RAGE, employing molecular docking and MD simulation approaches: Implications of potent therapeutic for diabetes and its related complications

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ABSTRACT

Diabetes mellitus is a global health challenge, ranking third among the mortality rates globally. Diabetic-mediated Advanced glycation end products (AGEs) associated with Receptor for Advanced Glycation End-products (RAGE) contribute to chronic diabetes and its complications, inflammatory, cancer, and neurodegenerative disorders. The information behind the binding mechanisms between AGEs-RAGE complexes remains elusive. In the current study, we used advanced computational approaches to reveal the intramolecular interactions of AGEs-RAGE which leads to multiple diseases. We have characterized AGEs-RAGE interactions by protein–ligand docking and molecular dynamic (MD) simulations were further conducted to evaluate the AGEs-RAGE complex stability. Subsequently, several residues emerged as pivotal in AGEs-RAGE complex formation. Further, MD simulation provides valuable insights into structural movements, stability, and conformational dynamics of protein–ligand complexes. Our findings underscore new insights into molecular mechanisms of AGEs-RAGE complex formation in diabetes and its related complications and the ease of the drug discovery process.

1. Introduction

Diabetes mellitus is a metabolic disease marked by elevated glucose levels in the blood referred to as hyperglycemia, a chronic metabolic condition triggered by impoverished insulin production or insulin resistance. Over the last three decades, the number of individuals living with diabetes mellitus has dramatically increased in countries with all income levels. About 422 million people worldwide have diabetes, with the vast majority living in middle- and low-income nations. In recent decades, there has been a consistent rise in both the incidence and

overall prevalence of diabetes [1,2]. The severe consequences of diabetes mellitus are nephropathy, amputations, peripheral neuropathy, autonomic neuropathy, joint pains, retinopathy, and atherosclerosis [3]. The possible mechanism behind these consequences includes vicious cycle of protein kinase C (PKC) activation, reactive oxygen species (ROS), and advanced glycation end products (AGEs) production [4]. AGEs are heterogeneous harmful compounds derived from non-enzymatic products that become oxidized and glycated [5]. They are mostly formed from exogenous sources such as high-fat diets or endogenous sources in an environment of hyperglycemia [6]. AGEs are

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Description of *Diduga siamensis* sp. nov. and newly recorded *D. scalprata* (Lepidoptera, Erebidae, Arctiinae) from Thailand, with a checklist of the genus *Diduga*

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Abstract

In this study, 11 species of the genus *Diduga* Moore, [1887] are recognized from Thailand, including a new species, *Diduga siamensis* Bayarsaikhan & Heppner **sp. nov.**, and one newly recorded species, *D. scalprata* Bayarsaikhan, Li & Bae, 2020. Illustrations of adults and genitalia of the additionally examined species are provided, with a checklist of known species of the genus from this country.

Key words: Lithosiini, description, taxonomy, distribution, new record, Oriental Region

Introduction

Diduga Moore, [1887] is one of the diverse genera of the tribe Lithosiini, subfamily Arctiinae. This genus is represented by 49 species, widely distributed in the South and Southeast Asian countries (Fang 2000, Holloway 2001, Černý & Pinratana 2009, Kishida 2011, Bucsek 2012, 2014, 2020, Zhao & Han 2020, Dubatolov & Bucsek 2016, Singh *et al.* 2021, Bayarsaikhan *et al.* 2023a, 2023b, 2024). During the examination of extensive Lithosiini materials deposited in the McGuire Center for Lepidoptera and Biodiversity (Florida) and the Osaka Prefecture University (Osaka) collected in Thailand, we found one undescribed species and one newly recorded species from Thailand.

In Thailand, 10 species of *Diduga* have been reported by Černý & Pinratana (2009), Bucsek (2012), and Bayarsaikhan *et al.* (2020). According to Bayarsaikhan *et al.* (2023a)'s checklist, one recorded species of *Diduga* in Thailand, *Diduga amoenusa* Bucsek, is placed into the genus *Tospitis* Walker. The purpose of this study is to describe an additional new species, *D. siamensis* Bayarsaikhan & Heppner **sp. nov.**, with report of one newly recorded species, *D. scalprata* Bayarsaikhan, Li & Bae, 2020, for this country. Consequently, 11 species of this genus are now known in Thailand.

Abbreviations for collections used: CKC—private collections of Karel Černý (CKC, Innsbruck, Austria); OPU = the Osaka Prefecture University, Osaka, Japan; MGCL = the McGuire Center for Lepidoptera and Biodiversity, Gainesville, USA.



ROSA26 BAC-based system enables continuous high-yield protein production

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Abstract

The mouse ROSA26 locus serves as a port to maintain stable expression of the transgene. Therefore, a bacterial artificial chromosome (BAC) containing the ROSA26 locus was used as a platform to produce proteins by incorporating transgenes with foreign promoters. However, the endogenous promoter of the ROSA26 locus, which allows stable high-level transgene expression, has not been applied to the ROSA26 BAC-based platform. In this study, we generated recombinant ROSA26 BAC by targeting “exon 1” or “intron 1” of the ROSA26 locus. Recombinant ROSA26 BAC (exon 1) had lower protein productivity compared to the control. However, recombinant ROSA26 BAC (intron 1) produced protein at a higher efficiency than the control group. The protein productivity induction effect by recombinant ROSA26 BAC (intron 1) was maintained up to a single clone, enabling stable protein production over a long period of time. Taken together, we established a ROSA26 BAC-based protein production system capable of producing protein at high yield over a long period of time.

Keywords ROSA26 BAC · Endogenous promoter · Intron 1

1 Introduction

One of the platforms used for protein production is based on plasmids [1]. Plasmid-based vectors continue to exist as extrachromosomally replicating episomes after transfection, enabling highest levels of gene expression between 24 and 96 h [1]. However, due to limited episomal stability, the expression level of the transgene gradually decreases [2–4]. To avoid these drawbacks, a process of selecting clones with desirable characteristics should be performed, which is both labor-intensive and time-intensive [5]. However, even after the establishment of a stably transfected clone, the chromatin

surrounding the transgenic integration site, have a significant impact on the expression of the transgene (i.e., positional chromatin effects) [6]. Based on these findings, there were doubts about the use of the widely used plasmid-based vector systems for protein production [7, 8]. A new vector platform called bacterial artificial chromosome (BAC) has been used as a way to address positional chromatin effects. A genomic region of 150–350 kb can be found in BAC, which contains all cis-acting regulatory elements (insulators, enhancers, promoters, etc.) [9]. Therefore, BAC is considered a safe expression unit that allows stable transgene expression without disturbing the nearby chromatin where integration occurs [10].

Through gene trapping, the ROSA26 locus was discovered on mouse chromosome 6 [11]. The ROSA26 locus maintained long-term gene expression and exhibited resistance to gene silencing, allowing the transgene to be expressed constitutively in vivo. To date, more than 560 knock-in lines at the ROSA26 locus have been generated in mouse [12]. ROSA26 BAC containing the ROSA26 locus also has been alternatively used to generate animal models [13–15]. The transgene inserted into the ROSA26 locus in the ROSA26 BAC accurately mimicked the original

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Article

Synergistic ROS Reduction Through the Co-Inhibition of BRAF and p38 MAPK Ameliorates Senescence

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Abstract: Reactive oxygen species (ROS)-mediated damage to macromolecules and cellular organelles is one of the major causes of senescence. Therapeutic strategies that lower ROS levels have been proposed as important treatments for senescence, but effective mechanisms for reducing ROS levels have not been discovered. Here, we aimed to find a combination that has a synergistic effect on ROS reduction using senomorphics known to reduce ROS. Combination treatment with BRAF inhibitor SB590885 and p38 MAPK inhibitor SB203580 showed a synergistic effect on ROS reduction compared to treatment with either drug alone. The synergistic effect of ROS reduction through this combination led to a synergistic effect that restored mitochondrial function and ameliorated senescence-associated phenotypes. To elucidate the underlying mechanism by which the synergistic effect of the two drugs reverses senescence, we performed RNA sequencing and identified *metallothionein 2A* (*MT2A*) as a key gene. *MT2A* was upregulated in response to combination therapy, and overexpression of *MT2A* led to a decrease in ROS and subsequent recovery of senescence-associated phenotypes, similar to the effects of combination therapy. Taken together, we found a drug combination that showed synergistic effects on ROS reduction, which contributed to the recovery of senescence-associated phenotypes through *MT2A* gene regulation. This study opens up a new avenue in aging research by demonstrating that combination therapy with existing senomorphics can enhance the ability to reverse senescence and that similar reversal effects can be achieved through gene regulation regulated by combination therapy.

Keywords: reactive oxygen species (ROS); BRAF inhibitor; p38 MAPK inhibitor; metallothionein 2A (*MT2A*)



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1. Introduction

Senescence is a biological process that occurs in all living organisms and is one of the inevitable phenomena. Changes in the structure and function of cellular organelles, especially the deterioration of mitochondria, are the hallmarks of senescence [1]. As senescence progresses, mitochondria undergo structural changes, which eventually lead to functional defects [2]. Damaged mitochondria release electrons in the electron transport complex (ETC) to produce ROS as byproducts [3]. Damaged mitochondria not only produce ROS but also become targets for damage caused by ROS [4]. Increased ROS levels further damage mitochondria, thereby upregulating mitochondrial ROS production. As ROS levels increase, the structure and function of other cellular organelles deteriorate,



One-step immunoassay of SARS-CoV-2 using screened Fv-antibodies and switching peptides

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ABSTRACT

The Fv-antibodies were corresponded to V_H region of immunoglobulin G, which were composed of three complementarity determining regions (CDRs) for the specific binding of antigens. In this work, the Fv-antibodies against SARS-CoV-2 spike protein (SP) were screened from an autotisplayed Fv-antibody library which was expressed on *E. coli* outer membrane, and the receptor binding domain (RBD) of SP was used as a screening probe. The screened target clones were analyzed to have quantitative binding properties to the RBD, and the Fv-antibodies from the screened target clones were expressed as soluble proteins. The binding affinity (K_D) of expressed Fv-antibodies to the RBD was estimated to be 70–85 nM using SPR biosensor. The specific binding properties of Fv-antibodies were analyzed for pseudo-virus particles with SARS-CoV-2 SP on the Lenti-virus envelope, such as wild type (Wuhan-1) and variants (Delta, Omicron BA.2, Omicron BA.4/5) using a SPR biosensor. The detection of real SARS-CoV-2 (Wild type, Wuhan-1) based on a SPR biosensor was also presented using the Fv-antibodies with the binding constant (K_D) of cycle threshold value (Ct) = 33.8–32.9 (2.19–4.08 copies/ μ L) and LOD of 0.67–0.83 copies/ μ L (Ct = 35.5–35.2). Finally, one-step immunoassay based on switching peptide was demonstrated for the detection of the real SARS-CoV-2 (Wuhan-1) without any washing step. The binding constant (K_D) was estimated to be Ct = 35.2–33.9 (0.83–2.04 copies/ μ L), and LOD was estimated to be 0.14–0.47 copies/ μ L (Ct = 37.8–36.0). Considering the LOD of the conventional RT-PCR (Ct = 35), the LOD of the one-step immunoassay based on the switching peptide was determined to be feasible for the medical diagnosis of COVID-19.

1. Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has triggered a global pandemic of COVID-19. Over the past two decades, three zoonotic coronaviruses have infected to human, such as SARS-CoV-1, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2. These viruses have the potential to cause severe acute respiratory distress syndrome (ARDS) to human (Lamers and Haagmans, 2022). These CoVs are known to produce four major types of proteins: envelope protein, membrane protein, nucleocapsid protein and spike protein (SP) (Zhang et al., 2021; Kim and Lee, 2022),

and SP has been extensively utilized in the development of vaccines and diagnostic assays (Amanat and Krammer, 2020; Martinez-Flores et al., 2021; Schubert et al., 2022). Especially, SARS-CoV-2 is known to infect host cells through the binding of SP on envelop of virus to angiotensin conversion enzyme 2 (ACE2) receptor on the outer membrane of host cell (Lan et al., 2020; Sun et al., 2020). The SP consists of two protein domains (S1 and S2), and the specific interaction with ACE2 receptor was especially occurred through the receptor binding domain (RBD) of SP which was located at the S1 domain of SP (Yang and Du, 2021). In this work, Fv-antibodies with the binding affinity to the RBD of SP was screened from Fv-antibody library, and the screened Fv-antibodies were

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Monoamine Oxidase-A (MAO-A) Inhibitors Screened from the Autodisplayed Fv-Antibody Library

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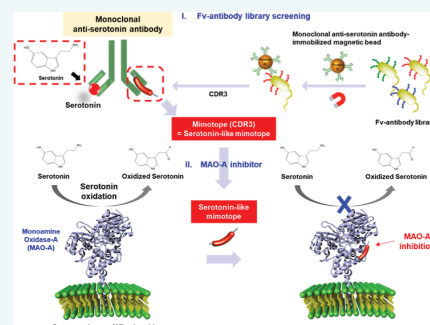
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Supporting Information

ABSTRACT: Serotonin-like mimotopes were screened from the Fv-antibody library to be used as inhibitors against monoamine oxidase A (MAO-A). The Fv-antibody [corresponding to the V_H region of immunoglobulin G (IgG)] consists of three complementarity-determining regions and four frame regions. The Fv-antibody library was prepared by site-directed mutagenesis of CDR3, which consists of 11 amino acid residues. Three target clones were screened from the Fv-antibody library, and the binding affinity of the screened clones to the monoclonal anti-serotonin antibody was analyzed using fluorescence-activated cell sorting. The screened Fv-antibodies were expressed as soluble proteins fused with green fluorescence protein. Additionally, the screened CDR3 regions (11 residues) of the selected Fv-antibodies were synthesized as peptides with linking amino acid residues. The binding constants (K_D) of the three serotonin-like mimotopes (Fv-antibodies and peptides) were estimated using a surface plasmon resonance biosensor. The inhibitory activity (IC_{50}) of the serotonin-like mimotopes (Fv-antibodies and peptides) was estimated separately for MAO-A and MAO-B enzymes and compared with that of conventional inhibitors. Finally, the screened serotonin-like mimotopes were used to treat a cell line (SH-SY5Y, ATCC code: CRL-2266) expressing serotonin receptors. This was done to confirm the following two aspects: (1) the binding of mimotopes to the serotonin receptors on the cell surface and (2) the inhibitory activity of mimotopes against MAO-A enzymes in the cell lysates.

KEYWORDS: Fv-antibody library, serotonin-like mimotope, monoamine oxidase-A (MAO-A), peptide



Monoamine oxidase A (MAO-A) and MAO-B are flavoenzymes located in the outer mitochondrial membrane. Both of these enzymes possess the cofactor flavin adenine dinucleotide (FAD) and exhibit over 70% homology in their amino acid sequence.^{1,2} Although MAO-A and MAO-B share high amino acid sequence homology, they have different substrate specificities and inhibitor sensitivities.³ MAO-A inhibitors are used to treat depression, anxiety, and migraines. MAO-B inhibitors have been used as dopamine-sparing agents for the treatment of Parkinson's disease.⁴ Being a neurotransmitter, serotonin is released into the synapse and subsequently reuptaken into the presynapse. MAO-A has been reported to disrupt the reuptake of serotonin by oxidizing it through the coenzyme (FAD).⁵ Inhibitors of MAO-A-like clorgiline have been used to maintain serotonin concentration.⁶ In this study, serotonin-like mimotopes were screened as inhibitors of MAO-A from an Fv-antibody library using a monoclonal anti-serotonin antibody.

The structure of the Fv-antibody is based on the V_H of immunoglobulin G (IgG), which is composed of three complementarity-determining regions (CDRs) and four frame regions (FRs).^{7–10} CDRs are known to be hypervariable regions located in the heavy and light chains of IgGs, which interact specifically with the target antigen. As shown in Figure 1a, the

Fv-antibody library was prepared with a randomized amino acid sequence of CDR3 through site-directed mutagenesis of 11 amino acid residues.^{11,12} The prepared Fv-antibody library was expressed on the outer membrane of *Escherichia coli* using autodisplay technology.¹³ As shown in Figure 1b, the autodisplay of target proteins was reported to achieve a high expression yield of $>10^5$ Fv-antibodies/*E. coli*; additionally, $>90\%$ of the *E. coli* cells within the total *E. coli* population in the Fv-antibody library autodisplayed the Fv-antibodies. The Fv-antibody library has been reported to exhibit a high diversity of $>10^5$ clones/library.¹⁴ It has been used to screen Fv-antibodies with binding affinity to biotin, fluorescent dyes (fluorescein and rhodamine B), biocrystals (monosodium urate and calcium pyrophosphate dehydrate), and viral proteins, among others.^{12,15–19}

In the previous work, the inhibitors of MAO-B (Fv-antibodies and peptides) were screened using the Fv-antibody library, and

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Two-dimensional segmentation fusion tool: an extensible, free-to-use, user-friendly tool for combining different bidimensional segmentations

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Introduction: In several fields, the process of fusing multiple two-dimensional (2D) closed lines is an important step. For instance, this is fundamental in histology and oncology in general. The treatment of a tumor consists of numerous steps and activities. Among them, segmenting the cancer area, that is, the correct identification of its spatial location by the segmentation technique, is one of the most important and at the same time complex and delicate steps. The difficulty in deriving reliable segmentations stems from the lack of a standard for identifying the edges and surrounding tissues of the tumor area. For this reason, the entire process is affected by considerable subjectivity. Given a tumor image, different practitioners can associate different segmentations with it, and the diagnoses produced may differ. Moreover, experimental data show that the analysis of the same area by the same physician at two separate timepoints may result in different lines being produced. Accordingly, it is challenging to establish which contour line is the ground truth.

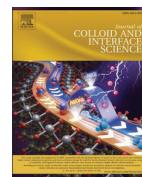
Methods: Starting from multiple segmentations related to the same tumor, statistical metrics and computational procedures could be exploited to combine them for determining the most reliable contour line. In particular, numerous algorithms have been developed over time for this procedure, but none of them is validated yet. Accordingly, in this field, there is no ground truth, and research is still active.

Results: In this work, we developed the *Two-Dimensional Segmentation Fusion Tool (TDSFT)*, a user-friendly tool distributed as a free-to-use standalone application for *MAC*, *Linux*, and *Windows*, which offers a simple and extensible interface where numerous algorithms are proposed to "compute the mean" (i.e., the process to fuse, combine, and "average") multiple 2D lines.



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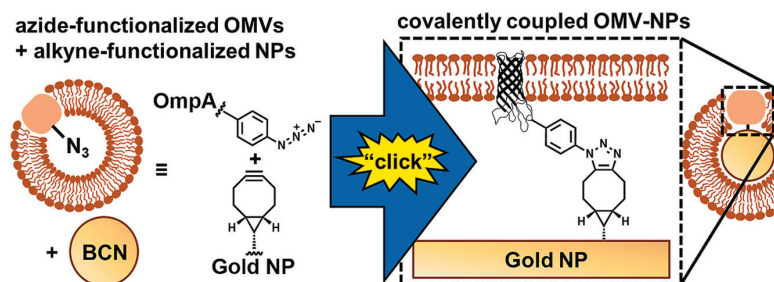
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Regular Article

Covalent coupling of functionalized outer membrane vesicles (OMVs) to gold nanoparticles

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GRAPHICAL ABSTRACT



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ABSTRACT

Outer membrane vesicle-functionalized nanoparticles (OMV-NPs) have attracted significant interest, especially regarding drug delivery applications and vaccines. Here, we report on novel OMV-NPs by applying bioorthogonal click reaction for encapsulating gold nanoparticles (NPs) within outer membrane vesicles (OMVs) by covalent coupling. For this purpose, outer membrane protein A (OmpA), abundant in large numbers (due to 100,000 copies/cell [1]) in OMVs, was modified via the incorporation of the unnatural amino acid p-azidophenylalanine. The azide group was covalently coupled to alkyne-functionalized NPs after incorporation into OmpA. A simplified procedure using low-speed centrifugation (1,000 x g) was developed for preparing OMV-NPs. The OMV-NPs were characterized by zeta potential, Laurdan-based lipid membrane dynamics studies, and the enzymatic activity of functionalized OMVs with surface-displayed nicotinamide adenine dinucleotide oxidase (Nox). In addition, OMVs from attenuated bacteria (ClearColiTM BL21 (DE3), *E. coli* F470) with surface-displayed Nox or antibody fragments were prepared and successfully coupled to AuNPs. Finally, OMV-NPs displaying single-chain variable fragments from a monoclonal antibody directed against epidermal growth factor receptor were applied to demonstrate the feasibility of OMV-NPs for tumor cell targeting.

Abbreviations: BCN-succinimidyl ester, (1R,8S,9S)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl N-succinimidyl carbonate; OMV, Outer membrane vesicle; NP, Nanoparticle; DBCO, dibenzylcyclooctyne; SPAAC, strain-promoted alkyne-azide cycloaddition; scFv, single-chain variable fragments.

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OPEN Impact of glucose metabolism on PD-L1 expression in sorafenib-resistant hepatocellular carcinoma cells

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Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer-related mortality worldwide. Programmed cell death ligand-1 (PD-L1) is an immune checkpoint protein that binds to programmed cell death-1 (PD-1), which is expressed in activated T cells and other immune cells and has been employed in cancer therapy, including HCC. Recently, PD-L1 overexpression has been documented in treatment-resistant cancer cells. Sorafenib is a multikinase inhibitor and the only FDA-approved treatment for advanced HCC. However, several patients exhibit resistance to sorafenib during treatment. This study aimed to assess the effect of glucose deprivation on PD-L1 expression in HCC cells. We used PD-L1-overexpressing HepG2 cells and IFN- γ -treated SK-Hep1 cells to explore the impact of glycolysis on PD-L1 expression. To validate the correlation between PD-L1 expression and glycolysis, we analyzed data from The Cancer Genome Atlas (TCGA) and used immunostaining for HCC tissue analysis. Furthermore, to modulate PD-L1 expression, we treated HepG2, SK-Hep1, and sorafenib-resistant SK-Hep1R cells with rapamycin. Here, we found that glucose deprivation reduced PD-L1 expression in HCC cells. Additionally, TCGA data and immunostaining analyses confirmed a positive correlation between the expression of hexokinase II (HK2), which plays a key role in glucose metabolism, and PD-L1. Notably, rapamycin treatment decreased the expression of PD-L1 and HK2 in both high PD-L1-expressing HCC cells and sorafenib-resistant cells. Our results suggest that the modulation of PD-L1 expression by glucose deprivation may represent a strategy to overcome PD-L1 upregulation in patients with sorafenib-resistant HCC.

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide and the most common cause of death in individuals with cirrhosis¹. Due to the asymptomatic nature of early-stage HCC, it is often only detected at intermediate or advanced stages, which reduces the effectiveness of curative treatments such as surgical resection, ablation, or liver transplantation². Sorafenib (Nexavar®), an orally-active multikinase inhibitor, is the first-line chemotherapy for advanced HCC. Clinical studies have shown that sorafenib can significantly extend the median survival time of patients with advanced HCC by approximately 3–5 months³. However, its anticancer efficacy is hindered by the development of drug resistance in the cells, resulting in a poor prognosis for patients^{4,5}. Therefore, it is important to restore susceptibility to sorafenib in patients with drug-resistant HCC.

Studies of the mechanisms underlying cancer immune evasion have focused on the programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway. PD-L1, a member of the B7 protein family, interacts with PD-1, which is expressed on various immune cells, such as antigen-presenting cells and endothelial cells^{6,7}. Activation of the PD-1/PD-L1 pathway leads to the suppression of immune cells and production of certain cytokines such as IFN- γ , resulting in the inhibition of the anti-tumor T cell response^{8,9}. High expression of PD-1 and PD-L1 is associated with poor prognosis, increased HCC aggressiveness, and a higher risk of tumor relapse in patients who have undergone curative resection^{10–13}. Nivolumab and pembrolizumab, two approved

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Combination of [^{18}F]FDG and [^{18}F]PSMA-1007 PET/CT predicts tumour aggressiveness at staging and biochemical failure postoperatively in patients with prostate cancer

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Abstract

Purpose [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography/computed tomography (PET/CT) has limitations in prostate cancer (PCa) detection owing to low glycolysis in the primary tumour. Recently, prostate-specific membrane antigen (PSMA) PET/CT has been useful for biochemical failure detection and radioligand therapy (RLT) guidance. However, few studies have evaluated its use in primary prostate tumours using PSMA and [^{18}F]FDG PET/CT. This study aimed to evaluate [^{18}F]PSMA-1007 and [^{18}F]FDG PET/CT for primary tumour detection and understand the association of metabolic heterogeneity with clinicopathological characteristics at staging and postoperatively.

Method This prospective study included 42 index tumours (27 acinar and 15 ductal-dominant) in 42 patients who underwent [^{18}F]PSMA-1007 and [^{18}F]FDG PET/CT and subsequent radical prostatectomy. All patients were followed for a median of 26 mo, and serum prostate-specific antigen levels were measured every 3 mo to evaluate biochemical failure. One-way analysis of variance, Tukey's multiple comparison test, and Fisher's exact test were performed.

Results All 42 index tumours were detected on [^{18}F]PSMA-1007 PET/CT, whereas only 15 were detected on [^{18}F]FDG PET/CT (62.3% vs. 37.7%, $p < 0.0001$). A high SUV_{max} for [^{18}F]PSMA-1007 was observed in tumours with high Gleason scores (GS 6–7 vs. GS 8–10; 12.1 vs. 20.1, $p < 0.05$). Tumours with [^{18}F]FDG uptake were mostly ductal dominant (acinar-dominant 4/27; ductal-dominant; 11/15, $p < 0.001$), with lower [^{18}F]PSMA-1007 uptake than tumours without [^{18}F]FDG uptake (SUV_{max} 16.58 vs. 11.19, $p < 0.001$). There were 16.6% (7/42) of patients with pStage IV in whom the primary tumours were [^{18}F]FDG positive. Biochemical failure was observed in 14.8% (4/27) of patients with [^{18}F]FDG negative tumours but in 53.3% (8/15) of patients with [^{18}F]FDG positive tumours ($p = 0.013$).

Conclusions [^{18}F]PSMA-1007 PET/CT was superior to [^{18}F]FDG PET/CT in detecting primary PCa. In contrast, tumours with [^{18}F]FDG uptake are associated with larger size, a ductal-dominant type, and likely to undergo metastasis at staging and biochemical failure postoperatively.

Keywords Biochemical failure · Cancer metabolism · Cancer staging · [^{18}F]FDG · [^{18}F]PSMA-1007 PET/CT · Prostate

Introduction

Prostate cancer (PCa) is one of the most common solid tumours in men and the second leading cause of cancer-related death worldwide [1]. The diagnosis of PCa involves digital rectal examination and prostate-specific antigen (PSA) blood test, followed by transrectal ultrasound- or

magnetic resonance imaging (MRI)-guided biopsy. MRI-guided biopsy can potentially improve the diagnosis and treatment [2]. This would be especially beneficial in men with prior negative biopsy results [3]. Thus, MRI has become the most favourable diagnostic modality for detecting clinically significant PCa. Unlike MRI, [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography/computed tomography (PET/CT) is limited in detecting PCa owing to its low glycolytic metabolism. This is attributed to the high citrate content in the prostate gland which inhibits glucose uptake by cancer cells. In contrast, PCa expresses a unique

Jisu Kim and Seunghwan Lee contributed equally to this work.

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Monocarboxylate transporter-1 (MCT-1) inhibitors screened from autodisplayed Fv-antibody library

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ABSTRACT

Monocarboxylate transporter-1 (MCT-1) inhibitors were screened from the Fv-antibody library, which contained complementary determining region 3 with randomized amino acid sequences (11 residues) through site-directed mutagenesis. Fv-antibodies against MCT-1 were screened from the autodisplayed Fv-antibody library. Two clones were screened, and the binding affinity (K_D) against MCT-1 was estimated using flow cytometry. The screened Fv-antibodies were expressed as soluble fusion proteins (Fv-1 and Fv-2) and the K_D for MCT-1 was estimated using the SPR biosensor. The inhibitory activity of the expressed Fv-antibodies was observed in HEK293T and Jurkat cell lines by measuring intracellular pH and lactate accumulation. The level of cell viability in HEK293T and Jurkat cell lines was decreased by the inhibitory activity of the expressed Fv-antibodies. The binding properties of the Fv-antibodies to MCT-1 were analyzed using molecular docking simulations. Overall, the results showed that the screened Fv-antibodies against MCT-1 from the Fv-antibody library had high binding affinity and inhibitory activity against MCT-1, which could be used as potential therapeutic drug candidates for the MCT-1 inhibitor.

1. Introduction

Cancer cells can derive energy through increased glycolysis, even in the presence of oxygen, a phenomenon referred to as the Warburg effect [1]. This metabolic alteration results in the accumulation of intracellular lactic acid, which must be exported to prevent cell death caused by cellular acidosis [2]. Furthermore, supplying lactate as a substrate to the microenvironment leads to tumor invasion and immune suppression in neighboring tumor cells [3]. Monocarboxylate transporters (MCTs, MCT1–4) are proton-linked plasma membrane proteins that play an essential role in cellular metabolism and pH regulation [4]. MCT-1 (SLC16A1) and MCT4 (SLC16A3) mainly carry out lactate uptake and efflux pathways [5,6]. Thus, overexpression of MCT1 and/or MCT4 has been reported in a variety of solid tumors. [7–9] In addition, activated T cells also increase lactate production for energy metabolism [10,11].

MCT-1 exhibits a high affinity for L-lactate ($K_m = 3.5\text{--}10\text{ mM}$), whereas MCT4 has a low L-lactate affinity ($K_m = 22\text{--}28\text{ mM}$) [5,6]. MCT1 blockade on tumor cells or T cells might induce the accumulation of intracellular lactate, rapidly disabling tumor cell growth and glycolysis.

Several inhibitors targeting MCT-1, including MCT-1 inhibitors 1) SR13800 (AR-C122982, pyrolytic pyrimidine-based molecules) [12,13], 2) AZD3965 [14,15], 3) BAY8002 [16] have been investigated. The MCT-1 inhibitor SR13800 effectively disrupts lactate homeostasis and reduces the proliferation of various cells, including B-cell lymphoma, breast cancer cells, and neuroblastoma cells [12,13]. The 50 % inhibitory concentration (IC_{50}) of cell proliferation after treatment with SR13800 in neuroblastoma and breast cancer cells ranged from 14.6 to 31.3 μM [13], whereas the IC_{50} for Raji Burkitt lymphoma cells was 5 nM [12]. AZD3965 is an orally bioavailable, MCT-1-specific inhibitor derived from AR-C155858 (MCT1/2 inhibitor). The IC_{50} values for growth

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ARTICLE OPEN



Acetyl-CoA synthetase 2 contributes to a better prognosis for liver cancer by switching acetate-glucose metabolism

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Acetyl-CoA synthetase 2 (ACSS2)-dependent acetate usage has generally been associated with tumorigenesis and increased malignancy in cancers under nutrient-depleted conditions. However, the nutrient usage and metabolic characteristics of the liver differ from those of other organs; therefore, the mechanism of ACSS2-mediated acetate metabolism may also differ in liver cancer. To elucidate the underlying mechanisms of ACSS2 in liver cancer and acetate metabolism, the relationships between patient acetate uptake and metabolic characteristics and between ACSS2 and tumor malignancies were comprehensively studied in vitro, in vivo and in humans. Clinically, we initially found that ACSS2 expression was decreased in liver cancer patients. Moreover, PET-CT imaging confirmed that lower-grade cancer cells take up more ¹¹C-acetate but less ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG); however, this trend was reversed in higher-grade cancer. Among liver cancer cells, those with high ACSS2 expression avidly absorbed acetate even in a glucose-sufficient environment, whereas those with low ACSS2 expression did not, thereby showing correlations with their respective ACSS2 expression. Metabolomic isotope tracing in vitro and in vivo revealed greater acetate incorporation, greater lipid anabolic metabolism, and less malignancy in high-ACSS2 tumors. Notably, ACSS2 downregulation in liver cancer cells was associated with increased tumor occurrence in vivo. In human patient cohorts, patients in the low-ACSS2 subgroup exhibited reduced anabolism, increased glycolysis/hypoxia, and poorer prognosis. We demonstrated that acetate uptake by ACSS2 in liver cancer is independent of glucose depletion and contributes to lipid anabolic metabolism and reduced malignancy, thereby leading to a better prognosis for liver cancer patients.

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INTRODUCTION

Glucose is considered the preferred nutrient for cancer cells according to Otto Warburg's proposal of aerobic glycolytic properties for all growing cancer cells¹. However, other nutrients, such as glutamine, lactate, and acetate, can also be utilized by tumors under certain environmental conditions^{2–4}. Acetate usage depends on acetyl-CoA synthetase 2 (ACSS2); specifically, the use of acetate in cancers seems to occur mostly under nutrient-depleted or hypoxic conditions^{4–6}. Another aspect of acetate uptake is its relationship with tumor malignancy. A higher level of ACSS2 is associated with a shorter survival time and metastasis in patients with brain, breast, or prostate cancers^{7,8}. Bioinformatic analyses have suggested links between increased acetate utilization and higher malignancy under hypoxic conditions^{9,10}.

Although the above studies suggest that hypoxia/nutrient depletion is positively correlated with cancer and that hypoxia/nutrient depletion and higher acetate uptake are positively correlated with cancer malignancy, there is contrasting evidence. It has been established that a high glucose uptake, which might limit acetate uptake, can be induced under hypoxic conditions via

hypoxia-inducible factors associated with increased cancer malignancy^{11,12}. Acetate metabolism through ACSS2 seems to be important in leukemogenesis, although blood cancer cells are exposed to nutrient- and oxygen-rich environments¹³. Positron emission tomography (PET) imaging has shown that well-differentiated liver cancer is better detected with ¹¹C-acetate, whereas poorly differentiated liver cancer is better detected with ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG)¹⁴. Therefore, there is a lack of clarity regarding the relationships between acetate uptake, nutrient availability and cancer malignancy; moreover, these relationships may differ based on cancer type and stage.

The nutrient usage and metabolic characteristics of the liver may differ from those of other organs, as exemplified by its synthesis of hepatic glucose and cholesterol relative to their consumption in other organs¹⁵. Given the unique metabolic nature of the liver and the controversies stated above, the relationship between acetate uptake, metabolic characteristics, and liver cancer malignancy cannot be extrapolated from other tumors and has yet to be fully elucidated. Therefore, in this study, we clarified the correlation between acetate metabolism and liver

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Enhancing the anticancer effect of androgen deprivation therapy by monocarboxylate transporter 1 inhibitor in prostate cancer cells

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Abstract

Background: Tumor initiation and progression necessitate a metabolic shift in cancer cells. Consequently, the progression of prostate cancer (PCa), a leading cause of cancer-related deaths in males globally, involves a shift from lipogenic to glycolytic metabolism. Androgen deprivation therapy (ADT) serves as the standard treatment for advanced-stage PCa. However, despite initial patient responses, castrate resistance emerges ultimately, necessitating novel therapeutic approaches. Therefore, in this study, we aimed to investigate the role of monocarboxylate transporters (MCTs) in PCa post-ADT and evaluate their potential as therapeutic targets.

Methods: PCa cells (LNCaP and C4-2 cell line), which has high prostate-specific membrane antigen (PSMA) and androgen receptor (AR) expression among PCa cell lines, was used in this study. We assessed the expression of MCT1 in PCa cells subjected to ADT using charcoal-stripped bovine serum (CSS)-containing medium or enzalutamide (ENZ). Furthermore, we evaluated the synergistic anticancer effects of combined treatment with ENZ and SR13800, an MCT1 inhibitor.

Results: Short-term ADT led to a significant upregulation in folate hydrolase 1 (*FOLH1*) and solute carrier family 16 member 1 (*SLC16A1*) gene levels, with elevated PSMA and MCT1 protein levels. Long-term ADT induced notable changes in cell morphology with further upregulation of *FOLH1*/PSMA and *SLC16A1*/MCT1 levels. Treatment with ENZ, a nonsteroidal anti-androgen, also increased PSMA and MCT1 expression. However, combined therapy with ENZ and SR13800 led to reduced PSMA level, decreased cell viability, and suppressed expression of cancer stem cell markers and migration indicators. Additionally, analysis of human PCa tissues revealed a positive correlation between PSMA and MCT1 expression in tumor regions.

Conclusions: Our results demonstrate that ADT led to a significant upregulation in MCT1 levels. However, the combination of ENZ and SR13800 demonstrated a promising synergistic anticancer effect, highlighting a potential therapeutic significance for patients with PCa undergoing ADT.

KEYWORDS

androgen deprivation therapy, anticancer effect, cancer metabolism, MCT1, PSMA

Visualizing cancer-originating acetate uptake through monocarboxylate transporter 1 in reactive astrocytes in the glioblastoma tumor microenvironment

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Abstract

Background. Reactive astrogliosis is a hallmark of various brain pathologies, including neurodegenerative diseases and glioblastomas. However, the specific intermediate metabolites contributing to reactive astrogliosis remain unknown. This study investigated how glioblastomas induce reactive astrogliosis in the neighboring microenvironment and explore ¹¹C-acetate PET as an imaging technique for detecting reactive astrogliosis.

Methods. Through in vitro, mouse models, and human tissue experiments, we examined the association between elevated ¹¹C-acetate uptake and reactive astrogliosis in gliomas. We explored acetate from glioblastoma cells, which triggers reactive astrogliosis in neighboring astrocytes by upregulating MAO-B and monocarboxylate transporter 1 (MCT1) expression. We evaluated the presence of cancer stem cells in the reactive astrogliosis region of glioblastomas and assessed the correlation between the volume of ¹¹C-acetate uptake beyond MRI and prognosis.

Results. Elevated ¹¹C-acetate uptake is associated with reactive astrogliosis and astrocytic MCT1 in the periphery of glioblastomas in human tissues and mouse models. Glioblastoma cells exhibit increased acetate production as a result of glucose metabolism, with subsequent secretion of acetate. Acetate derived from glioblastoma cells induces reactive astrogliosis in neighboring astrocytes by increasing the expression of MAO-B and MCT1. We found cancer stem cells within the reactive astrogliosis at the tumor periphery. Consequently, a larger volume of ¹¹C-acetate uptake beyond contrast-enhanced MRI was associated with a worse prognosis.

Conclusions. Our results highlight the role of acetate derived from glioblastoma cells in inducing reactive astrogliosis and underscore the potential value of ¹¹C-acetate PET as an imaging technique for detecting reactive astrogliosis, offering important implications for the diagnosis and treatment of glioblastomas.

Key Points

- ¹¹C-acetate PET imaging visualizes reactive astrogliosis in the periphery of the glioma.
- Acetate, derived from glioma cells, triggers reactive astrogliosis in neighboring astrocytes.
- The volume of reactive astrogliosis of glioma is associated with patient prognosis.



Surface Plasmon Resonance (SPR) Biosensor for the Detection of SARS-CoV-2 Using Autodisplayed F_V-antibodies on Outer Membrane of *E. coli*

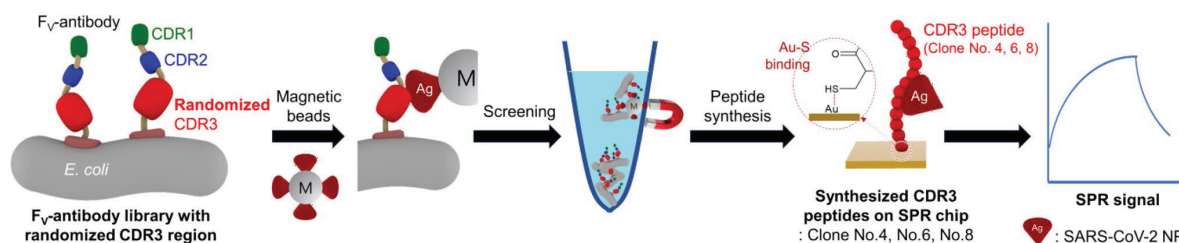
Ji-Hong Bong^{1,2,6} · Soo Jeong Lee¹ · Jaeyong Jung¹ · Jeong Soo Sung¹ · Min-Jung Kang³ · Misu Lee^{4,6} · Joachim Jose⁵ · Jae-Chul Pyun¹

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein (NP) participates in viral genome packaging and abundantly produced when infected. In this work, SPR biosensor for the detection of SARS-CoV-2 in viral fluid using F_V-antibodies with the binding affinity to nucleocapsid protein (NP) of SARS-CoV-2. The F_V-antibodies with a specific binding activity to the SARS-CoV-2 NP were screened using the F_V-antibody library, which was expressed on the outer membrane of *E. coli*. F_V-antibodies comprised three complementarity-determining regions (CDRs) and four frame regions (FRs) of the heavy chain at the binding pocket of IgG. The F_V-antibody library was prepared by performing site-directed mutagenesis and by using the autodisplay technology; F_V-antibodies with specific binding activities to the nucleocapsid protein (NP) of SARS-CoV-2 were screened using NP-immobilized magnetic beads. First, *E. coli* isolates with the target F_V-antibody were screened, and the binding affinity (K_D) was estimated for the screened *E. coli* clones using FACS analysis. Then, the outer membrane (OM) of the screened *E. coli* clones with autodisplayed F_V-antibodies was obtained and layered on an SPR biosensor, and the binding curves of four different coronavirus (CoV) culture fluids, SARS-CoV-2, SARS-CoV, MERS-CoV, and CoV strain 229E, were compared. Finally, the F_V-antibodies of the screened *E. coli* clones were synthesized as peptides (11 amino acid residues), and the binding constants (K_D) to NP as well as the binding curves of the CoV strains in culture fluids were estimated. Using docking simulation, binding sites and interaction types between NP and each synthetic peptide were investigated.

Graphical Abstract



Keywords F_V-antibody library · SARS-CoV-2 nucleocapsid protein (NP) · Autodisplay · Surface plasmon resonance · Docking simulation

Ji-Hong Bong and Soo Jeong Lee contributed equally to this work

Extended author information available on the last page of the article

Epidermal Growth Factor Receptor (EGFR) Inhibitors Screened from Autodisplayed Fv-Antibody Library

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Jeong Soo Sung, Jaeyong Jung, Tae-Hun Kim, Soonil Kwon, Hyung Eun Bae, Min-Jung Kang, Joachim Jose, Misu Lee, and Jae-Chul Pyun*



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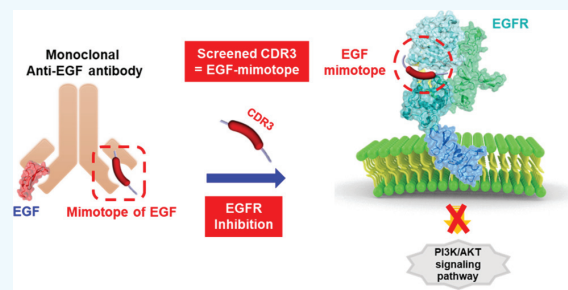
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ABSTRACT: Inhibitors of the epidermal growth factor receptor (EGFR) were screened from an autodisplayed Fv-antibody library using an anti-EGF antibody. The Fv-antibody library was expressed on the outer membrane of *Escherichia coli*, which corresponds to the heavy chain V_H region of immunoglobulin G. The library was constructed by randomizing the CDR3 region of expressed V_H regions (11 amino acid residues) by site-directed mutagenesis. Using an anti-EGF antibody as a screening probe, amino acid sequences (CDR3 region) with antibody binding affinity were screened from the Fv-antibody library. These amino acid sequences were considered to have similar chemical properties to EGF, which can bind to EGFR. Two autodisplayed clones with Fv-antibodies against EGFR were screened from the Fv-antibody library, and the screened Fv-antibodies were expressed as soluble proteins. The binding affinity (K_D) was estimated using an SPR biosensor, and the inhibitory activity of expressed Fv-antibodies was observed for PANC-1 pancreatic tumor cells and T98G glioblastoma cells using Western blot analysis of proteins in the EGFR-mediated signaling pathway. The viability of PANC-1 and T98G cells was observed to decrease via the inhibitory activity of expressed Fv-antibodies. Finally, interactions between Fv-antibodies and EGFR were analyzed by using molecular docking simulations.



1. INTRODUCTION

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein that consists of an extracellular ligand-binding domain, a hydrophobic transmembrane region, and a cytoplasmic tyrosine kinase domain. EGFR interacts with various ligands, including epidermal growth factor (EGF), transforming growth factor α (TGF- α), and heparin-binding EGF-like growth factor (HB-EGF).¹ The activation of EGFR initiates a complex cascade, beginning with ligand binding, that induces phosphorylation of the cytoplasmic domain. This intricate process triggers a series of events, including the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway in signal transduction.^{2–4} Concomitant with activation of the ligand-binding signaling pathway, EGFR undergoes internalization and proceeds to either degradation or recycling.⁵ The EGF-induced signaling pathway is implicated in promoting growth, invasion, and metastasis of various tumors, leading to unfavorable clinical outcomes.⁶ Therefore, EGFR has emerged as a critical target for cancer therapy, encompassing nonsmall-cell lung cancer, glioma, pancreatic cancer, and breast cancer.⁷

EGFR inhibitors are classified into two categories: small-molecule tyrosine kinase inhibitors (TKIs) that bind to the

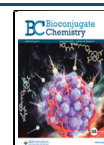
intracellular catalytic domain of EGFR and inhibitors that bind to the extracellular domain of EGFR, thus hindering receptor dimerization. First-generation anticancer drugs such as Gefitinib bind to the ATP-binding site within the intracellular domain of EGFR.^{8,9} However, resistance typically arises within 9–14 months in Gefitinib-treated patients, with the majority exhibiting the T790 M mutation involving tyrosine kinase domain exon 20.¹⁰ Second- and third-generation EGFR-TKIs have been developed to overcome mutations induced by first-generation agents. Afatinib and dacomitinib irreversibly inhibit the tyrosine kinase domain of EGFR; in particular, Afatinib inhibits the activity of EGFR T790 M. Osimertinib, a third-generation EGFR-TKI, also effectively targets EGFR T790 M by binding to the cysteine at C797 within the ATP-binding site of EGFR.¹¹

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Preventing SARS-CoV-2 infection using Fv-antibodies targeting the proprotein convertase (PPC) cleavage site

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Fv-antibodies targeting the proprotein convertase (PPC) region of the SARS-CoV-2 spike protein (SP) were screened from an Fv-antibody library to inhibit SARS-CoV-2 infection. Two selected Fv-antibodies were expressed as soluble recombinant proteins, and their binding affinities were assessed using a surface plasmon resonance biosensor. The binding regions of these Fv-antibodies corresponded to the cleavage sites of furin (S1/S2) and transmembrane serine protease 2 (TMPRSS2, S2'). The neutralizing activities of the two Fv-antibodies were demonstrated using a cell-based infection assay with pseudo-viruses carrying the SP of four different SARS-CoV-2 variants: wild-type (D614), delta (B.1.617.2), omicron (BA.2), and omicron (BA.4/5).

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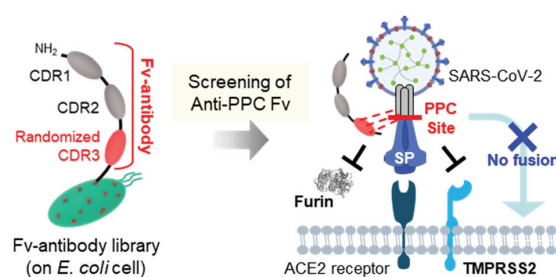
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1. Introduction

The infection process of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) begins with the binding of the spike protein (SP) to the angiotensin-converting enzyme 2 (ACE2) receptor.^{1–3} For SARS-CoV-2 to further infect the host cell membrane, the spike protein must be cleaved at two regions known as S1/S2 (residues: 682–685) and S2' (residues: 809–815), referred to as proprotein convertase (PPC) sites.^{4,5} The proteolytic enzymes furin (S1/S2) and transmembrane serine protease 2 (TMPRSS2, S2') are responsible for cleaving these sites, respectively.^{6,7} Due to the highly conserved amino acid sequences in these regions among coronaviruses, antibodies targeting these sites have been developed to prevent SARS-CoV-2 infection.^{8–11} However, accessing these regions with antibodies is challenging due to steric hindrance from the spike protein structure around the PPC region.¹² To overcome the steric hindrance in accessing the PPC region, many research groups have developed small molecules with binding affinity to these sites as pan-corona drugs.^{13–15} In this study, Fv-antibodies with high binding affinity to the PPC region were screened from an Fv-antibody library to prevent

SARS-CoV-2 infection, as illustrated in Scheme 1. Fv-antibodies are derived from the V_H region of immunoglobulin G (IgG), which consists of three complementarity-determining regions (CDR1–3) and four framework regions (FR1–4).^{16,17} The Fv-antibody library was produced by randomizing CDR3 (11 amino acid residues) through site-directed mutagenesis. The prepared Fv-antibody library was expressed on the outer membrane of *E. coli* using autodisplay technology.^{3,10,18–21}

This method utilizes the AIDA-1 to transport the expressed target proteins through its beta-barrel.²² The prepared Fv-antibody library exhibited a high surface density of 10⁵ Fv-antibodies per *E. coli* and a diversity of 10⁶ Fv-antibodies per library. The Fv-antibodies were screened using the amino acid sequence of the PPC region (residue: 661–900), which includes two cleavage sites, as the screening probe. The selected Fv-antibodies were expressed as soluble proteins, and their binding activities to the PPC region (S1/S2 and S2', respectively) were evaluated. Finally, the neutralizing activity



Scheme 1 Fv-antibodies targeting the PPC region of SARS-CoV-2 to prevent infection, screened from an Fv-antibody library.

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Reduction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant infection by blocking the epidermal growth factor receptor (EGFR) pathway

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AUTHOR AFFILIATIONS See affiliation list on p. 11.

ABSTRACT The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants presents challenges in global efforts to transition from the pandemic to an endemic stage. The spike protein of the SARS-CoV-2 virus, which is pivotal for cell entry, exhibits significant mutations in its variants, potentially affecting infectivity and therapeutic efficacy. Recent findings indicate upregulation of the epidermal growth factor receptor (EGFR) pathway, a key target in cancer therapy, by the spike protein of SARS-CoV-2. This study aimed to investigate the activity of the EGFR pathway against SARS-CoV-2 variants and to assess the inhibitory effects of EGFR inhibitors using SARS-CoV variant pseudoviral particles to guide future therapeutic strategies. Omicron variant SARS-CoV pseudoviral particles exhibited heightened infectivity in human angiotensin-converting enzyme 2 (hACE2)-expressing HEK293 and A549 lung cancer cells accompanied by increased EGFR pathway activation in infected cells. Using the EGFR tyrosine kinase inhibitor, osimertinib, we observed a reduction in viral infection rates in hACE2-HEK293 and A549 cells infected with the SARS-CoV-2 variant pseudoviral particles. We conducted further experiments to confirm that the reduction in infection efficacy with osimertinib treatment was not associated to a decrease in cell viability. Furthermore, this inhibitory effect of osimertinib in cell lines was corroborated in a spheroid cell culture model derived from hACE2-A549 cells. These findings suggest the potential application of EGFR-targeted antiviral therapy against highly infectious SARS-CoV-2 variants.

IMPORTANCE The emergence of novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants is concerning as vaccines designed for one variant need not essentially protect against other novel variants. Therefore, there is an urgent need to identify therapies that can act against multiple novel variants that have heightened virulence compared with the wild type. It has been reported that the spike protein of the SARS-CoV-2 virus elicits an increased expression of the epidermal growth factor receptor (EGFR) pathway. We used this information and examined whether treatment with an EGFR inhibitor, osimertinib, which is already approved for clinical use in cancer therapy, can reduce the infection caused by SARS-CoV-2, wild type, and Omicron and Delta variants, in two cell lines and one spheroid model. The results showed that osimertinib treatment successfully reduced infection efficacy, particularly in variants, and that this effect was not related to a reduction in cell viability, making this a promising strategy for treating SARS-CoV-2 infections.

KEYWORDS SARS-CoV-2 variants, spike protein mutations, EGFR pathway, osimertinib, antiviral therapy

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Yeonju Han and Seunghwan Kim contributed equally to this article. The author order was determined by their contribution to the article.

The authors declare no conflict of interest.

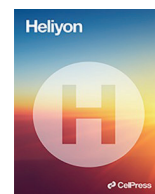
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Research article

Anticancer effects of high-dose extracellular citrate treatment in pancreatic cancer cells under different glucose concentrations

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ARTICLE INFO

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive solid tumor. Recently, the uptake of extracellular citrate by the sodium-dependent citrate transporter (NaCT), encoded by *SLC13A5*, has been demonstrated to exert profound effects on cancer cell metabolism. However, research on the function of extracellular citrate in PDAC pathogenesis and the relationship between NaCT expression and the tumor metabolic microenvironment is limited. Therefore, we aimed to evaluate the expression of citrate transporters across a spectrum of glucose concentrations in pancreatic cancer and systematically explore the effects of sodium citrate treatment on pancreatic cancer cells at different glucose concentrations. We observed a positive correlation between glucose concentration and NaCT expression in PDAC cell lines. Extracellular sodium citrate significantly reduced cell viability partially due to reduction in intracellular Ca^{2+} levels and decreased the migration of human PDAC cells. Furthermore, we observed a decrease in the levels of the stem cell marker prominin I (CD133) following sodium citrate treatment. Notably, the combination treatment of gemcitabine and extracellular sodium citrate exhibited a synergistic anticancer effect in both two-dimensional (2D) and three-dimensional (3D) culture systems. Additionally, we confirmed that pH slightly increased upon administration of sodium citrate, indicating that this could potentially augment the efficacy of gemcitabine. Altogether, these findings suggest that exogenous sodium citrate treatment, particularly in combination with gemcitabine, may represent a novel therapeutic strategy for treating PDAC. This approach holds promise for disrupting PDAC cell metabolism and inhibiting tumor progression.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC), an aggressive solid tumor, is the fourth leading cause of global cancer-related deaths [1, 2]. Despite the introduction of innovative therapeutic strategies, PDAC prognosis remains poor, with a 5-year survival rate of less than 5% and a median survival duration of approximately 6 months [3]. Surgical resection is often not viable for advanced PDAC; therefore, gemcitabine (genotoxic DNA-damaging agent) is the primary treatment option. However, gemcitabine often elicits a transient, minimal, or no response in most patients with unresectable PDAC, leading to therapeutic resistance, metastatic progression, and increased

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Screened Fv-Antibodies against the Angiotensin-Converting Enzyme 2 (ACE2) Receptor Neutralizing the Infection of SARS-CoV-2

Jaeyong Jung, Soonil Kwon, Jeong Soo Sung, Hyung Eun Bae, Min-Jung Kang, Joachim Jose, Misu Lee,* and Jae-Chul Pyun*



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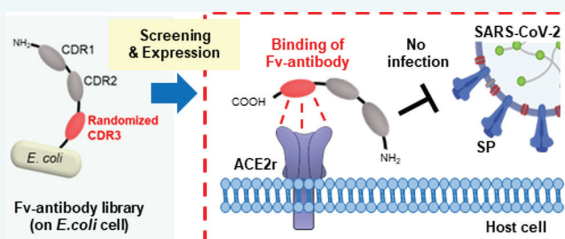
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Supporting Information

ABSTRACT: For the prevention of SARS-CoV-2 infection, four Fv-antibodies with binding affinity for the ACE2 receptor were screened from an Fv-antibody library. The screened Fv-antibodies were expressed as soluble proteins and estimated to have a high binding affinity, comparable to that between SARS-CoV-2 and the ACE2 receptor. The interaction between the Fv-antibodies and the ACE2 receptor was analyzed using docking simulation, and the significant binding affinity of the screened Fv-antibodies was attributed to the homology in amino acid sequence with the ACE2 receptor. The neutralizing activities of the Fv-antibodies were demonstrated using a cell-based infection assay based on four pseudo-virus types with SARS-CoV-2 variant spike proteins (Wild-type D614, Delta B.1.617.2, and Omicron BA.2, and Omicron BA.4/5).

KEYWORDS: SARS-CoV-2, ACE2 receptor, Fv-antibody library, neutralizing activity, cell-based infection assay, pseudo-virus particle



Severe acute respiratory coronavirus 2 (SARS-CoV-2) is an RNA virus with a 30 kb genome that encodes a replicase and four structural proteins: spike protein (SP), envelope protein (EP), membrane protein (MP), and nucleocapsid protein (NP).^{1–4} The receptor-binding domain (RBD) of SARS-CoV-2 SP is responsible for viral infection by binding to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell.⁵ The ACE2 receptor is present on epithelial cells of the digestive and respiratory systems in both humans and animals as well as in the vascular and excretory systems.⁶ Inhibition of the ACE2 receptor is known to be an essential mechanism to block viral entry into host cells.^{7–9} Two effective strategies have been the use of anti-ACE2 monoclonal antibodies (mAbs) and ACE2 receptor decoys, both of which target the interaction between SARS-CoV-2 and the ACE2 receptor. Anti-ACE2 mAbs directly bind to the ACE2 receptor on host cells, which prevents the initial step of viral entry.¹⁰ On the other hand, ACE2 receptor decoys replicate the structural features of the ACE2 receptor, which corresponds particularly to the RBD-binding region.^{11,12} These recombinant ACE2 decoys can neutralize the virus by providing an alternative binding site. By competing with ACE2 receptors on host cells for virus binding, these decoys effectively prevent SARS-CoV-2 SP from interacting with host cells. The methods to inhibit SARS-CoV-2 infection through the binding of SP to the ACE2 receptor were compared with other approaches including phage library, immunization, and ACE2 decoys, as summarized in Table 1. In this study, Fv-antibodies targeting the ACE2 receptor were

Table 1. Comparison of Inhibition Methods of SARS-CoV-2 Infection through the ACE2 Receptor

application	inhibitor	affinity (nM)	reference
phage library	peptide	543.0	27
sample of patients	IgM autoantibody	5.6–21.7	28
immunization	IgG antibody	11.2	29
immunization	IgG antibody	0.6	10
ACE2r mutant library	ACE2r display	17.6	30
ACE2r mutant library	ACE2r decoy	0.6–22.0	11
ACE2r mutant library	ACE2r decoy	5.4	12
Fv-antibody library	Fv-antibody	26.0	This work

screened from an Fv-antibody library. The binding affinity of these Fv-antibodies was found to be comparable to that of other methods, according to the reported binding affinity between SARS-CoV-2 SP and the ACE2 receptor (ranging from 4.7 to 10.0 nM).^{13–17}

Additionally, their ability to neutralize SARS-CoV-2 pseudo-virus particles expressing the variant SP (Wild, Delta, and Omicron variants)^{12,18} was evaluated. This assessment involved measuring their capacity to block the virus from

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One-step ultra-rapid immunoassay of calcitonin gene-related peptide for migraine diagnosis

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ABSTRACT

Migraine is known to be caused by calcitonin gene-related peptide (CGRP), prompting the need for quantitative analysis of CGRP for the clinical treatment of monoclonal antibodies targeting CGRP. Since CGRP is cleaved by proteolytic enzymes post-blood collection, rapid analysis methods are required. In this study, a one-step immunoassay for CGRP was developed using chemically mimicking peptides (mimotopes) with an analysis time of 32 min. Four clones from an Fv-antibody library were screened using two types of monoclonal antibodies against CGRP. Mimotopes for each monoclonal antibody were synthesized into peptides of 15 residues. The binding affinity (K_D) was estimated, and the interaction with monoclonal antibodies was analyzed using docking simulations. Finally, a one-step immunoassay for CGRP was demonstrated using migraine patient samples ($n = 57$) and healthy volunteer controls ($n = 18$). The limit of detection (LOD) of one-step immunoassay based on Fremanezumab (mimotope F1) was estimated to be 8.8 pg/mL with the limit of quantification (LOQ) of 125.9 pg/mL. And, the one-step immunoassay based on Galcanezumab (mimotope G7) showed the LOD of 9.4 pg/mL and the LOQ of 84.7 pg/mL. The total analysis time was estimated to be approximately 32 min and the assay results were estimated to be statistically consistent with conventional CGRP assay.

1. Introduction

Calcitonin gene-related peptide (CGRP) is a member of the calcitonin peptide family, which includes calcitonin, amylin, adrenomedullin, and calcitonin-receptor-stimulating peptide (Durham, 2006; Edvinsson et al., 2018; Sekiguchi, 2018). CGRP, a neuropeptide composed of 37 amino acid residues (3.97 kDa), is produced in various peripheral and central neurons, primarily located in the trigeminal ganglion. CGRP exists in two isoforms, α -CGRP and β -CGRP, distinguished by three amino acids (Chiang and Schwedt, 2020). Among these, α -CGRP is the primary target for migraine treatment, found in both the peripheral and central nervous systems, while β -CGRP, located in the enteric nervous system, is less studied (Amara et al., 1982; Park and Kim, 2020).

Numerous studies have established CGRP as a crucial neuropeptide in migraine pathophysiology (Durham and Russo, 1999; Edvinsson and

Warfvinge, 2019; Goadsby et al., 1990). CGRP plays a pivotal role by inducing vasodilation, triggering neurogenic inflammation, and transmitting pain signals (Park and Kim, 2020). The CGRP receptor comprises three components: calcitonin-like receptor (CLR), receptor component protein (RCP), and receptor activity-modifying protein 1 (RAMP1). The binding of CGRP to its receptor initiates cellular signal transduction pathways, leading to vasodilation (Silberstein, 2013; Silberstein et al., 2007). This binding initiates CGRP receptors on trigeminal neurons, glial cells, immune cells, endothelial cells, and smooth muscle activate vasodilation, mast cell degranulation, neurogenic inflammation, and sensitization in the peripheral and central nervous systems. This abnormal activation or sensitization of the trigeminovascular system ultimately contributes to migraine (Chiang and Schwedt, 2020; Yuan et al., 2019).

Several studies have shown increased CGRP concentrations in the

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SARS-CoV-2 vaccine based on ferritin complexes with screened immunogenic sequences from the Fv-antibody library

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In this study, the vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was developed using ferritin complexes with the immunogenic sequences screened against the SARS-CoV-2 spike protein (SP) from the Fv-antibody library. The Fv-antibody library was prepared on the outer membrane of *E. coli* by the expression of the V_H region of immunoglobulin G (IgG) with a randomized complementarity-determining region 3 (CDR3). Four Fv-antibodies to the receptor-binding domain (RBD) were screened from the Fv-antibody library, which had a comparable binding constant (K_D) between SARS-CoV-2 SP and the angiotensin-converting enzyme 2 (ACE2) receptor. The binding sites of screened Fv-antibodies on the RBD were analyzed using a docking analysis, and these binding sites were used as immunogenic sequences for the vaccine. The four immunogenic sequences were modified and co-expressed as a part of ferritin which was assembled into a ferritin complex. After the vaccination of ferritin complexes to mice, the anti-sera were analyzed to have a high enough titer. Additionally, the immune responses were found to be activated by vaccination, such as the expression of IgG subclasses and the increased level of cytokines. The neutralizing activity of the anti-sera was estimated using a cell-based infection assay based on pseudo-virus expressing the SP of SARS-CoV-2 variants.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and vaccination has been used as an effective and long-term protective method to prevent SARS-CoV-2 infection.^{1,2} As SARS-CoV-2 is composed of four main structural proteins, including spike protein (SP), nucleocapsid protein (NP), membrane protein (MP), and envelope protein (EP),^{3,4} the receptor binding domain (RBD) of SP has been most frequently used as a target for vaccination, as it directly interacts with the angiotensin-converting enzyme 2 (ACE2) receptor of host cells during SARS-CoV-2 infection.^{5–7} As an effective vaccination

platform, the protein-based vaccines have been developed in combination with various adjuvants to enhance humoral immune responses. They activate toll-like receptor (TLR) and pattern recognition receptor (PRR) signaling pathways, promoting the maturation and activation of antigen-presenting cells (APCs) by stimulating antigen signals.^{8,9} For example, adjuvants such as aluminum oxide,¹⁰ other inorganic materials,¹¹ and nano-engineered coordination polymers¹² bind to antigens to enhance the vaccination efficiency. These adjuvants boost immunogenicity by promoting sustained antigen release, efficiently delivering antigens to specific targets, and inducing specific immune responses.¹¹ This activation leads to the production of cytokines.¹³ In particular, carrier proteins and biomolecules have been conjugated to immunogenic proteins or peptides, such as keyhole limpet hemocyanin (KLH),^{14–17} virus-like particles (VLPs),^{5,18,19} tetanus toxoid (TT),^{20,21} and diphtheria toxin.^{22–24} Along with protein-based vaccines, protein particle-based vaccines have been reported to be more readily taken up by APCs, and multivalent antigen presentation has been favorable for promoting receptor clustering and B cell activation.^{25–28} Ferritin is frequently used as a protein particle in vaccine development.^{29–31} It is a major iron storage protein

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Mucosal TLR5 activation controls healthspan and longevity

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Addressing age-related immunological defects through therapeutic interventions is essential for healthy aging, as the immune system plays a crucial role in controlling infections, malignancies, and in supporting tissue homeostasis and repair. In our study, we show that stimulating toll-like receptor 5 (TLR5) via mucosal delivery of a flagellin-containing fusion protein effectively extends the lifespan and enhances the healthspan of mice of both sexes. This enhancement in healthspan is evidenced by diminished hair loss and ocular lens opacity, increased bone mineral density, improved stem cell activity, delayed thymic involution, heightened cognitive capacity, and the prevention of pulmonary lung fibrosis. Additionally, this fusion protein boosts intestinal mucosal integrity by augmenting the surface expression of TLR5 in a certain subset of dendritic cells and increasing interleukin-22 (IL-22) secretion. In this work, we present observations that underscore the benefits of TLR5-dependent stimulation in the mucosal compartment, suggesting a viable strategy for enhancing longevity and healthspan.

Age-associated immunological alterations are multifaced and include enhanced susceptibility to infection and chronic tissue inflammation. Such alterations may contribute to the development of metabolic and cardiovascular diseases, neurodegenerative syndromes, and age-related ailments such as frailty^{1–3}. The effects of aging on the immune system appear at various levels, including decreased production of immune cells in the bone marrow and thymus, reduced function of mature lymphocytes in secondary lymphoid tissue, and decreased function of innate immune cells in various tissues⁴. Aging-dependent deterioration of innate immune cells includes the reduction in phagocytic activity, ROS generation, and reduction of pathogen recognition receptors (PRRs) expression, failing to defend against various infections and respond to vaccines⁵. Impaired functions of

innate immunity can further exacerbate the flaws in adaptive immunity, for instance, by not providing efficient antigen presentation to T cells⁶. In addition to influenza vaccines, recently, it has been reported that the effectiveness of vaccines against infections such as COVID-19 is reduced in the elderly. Following the BNT162b2 (Pfizer-BioNTech) coronavirus disease 2019 (COVID-19) vaccine, older adults (i.e., ≥80 years old) exhibited decreased IgG antibody titers against an antigen or neutralizing antibody production compared to younger adults (<60 years old)⁷. Since robust innate immune responses are critical for a strong vaccine response, adjuvants for vaccine target PRRs to boost a sufficient inflammatory innate immune response in the elderly.

Toll-like receptors (TLRs) are the major PRRs that induce innate immunity by recognizing pathogen-associated molecular patterns

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Article

Rapid and Accurate Ecotoxicological Assessment of Heavy Metals Using *Cyprinus carpio* Cells

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Abstract: Heavy metals have serious negative effects on various aquatic organisms, and therefore rapid and accurate ecotoxicological assessments of heavy metals are necessary. Fish-derived cells sensitive to heavy metals have been used as valuable tools for ecotoxicological assessments. However, this method requires a minimum toxicity treatment time of 96 h, which limits its use when rapid ecotoxicological assessments are required or ecotoxicological assessments of a large number of toxicants are performed. In this study, these limitations were overcome by adjusting parameters including the concentration of fetal bovine serum (FBS) in the medium and the treatment time of the toxicant. Specifically, we found that the maximum time for fish cells to remain unstarved was 6 h when using a medium containing 1% FBS. We applied both parameters to the ecotoxicological assessment (using a medium containing 1% FBS for the toxicity assessment and treating the toxicant for only 6 h). Surprisingly, these adjusted parameters allowed us to obtain faster and more accurate data than the traditional assessment. This improvement was due to the new assessment conditions that minimized the possibility that the growth-inducing effects of nutrients present in excess in the medium could interfere with the cellular response to the toxicant. The accuracy of this assessment was not limited to measuring the toxicity of heavy metals. In conclusion, we have established an ecotoxicity assessment that can generate rapid and accurate data on heavy metals. This new platform will become the cornerstone of rapid and accurate ecotoxicity assessments of heavy metals.

Keywords: rapid ecotoxicological assessment; accurate ecotoxicological assessment; *Cyprinus carpio*



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1. Introduction

Heavy metals have significant negative effects on various aquatic plants and animals. Continuous exposure to heavy metals gradually increases the concentration of toxic substances in the body of aquatic organisms. The toxic substances accumulated in the body can easily affect larvae and embryos, causing spinal deformation, morphological changes, decreased cardiac activity, and increased mortality. Heavy metals not only have negative

Article

Senescence Rejuvenation through Reduction in Mitochondrial Reactive Oxygen Species Generation by *Polygonum cuspidatum* Extract: In Vitro Evidence

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Abstract: Oxidative stress caused by reactive oxygen species (ROS) is one of the major causes of senescence. Strategies to reduce ROS are known to be important factors in reversing senescence, but effective strategies have not been found. In this study, we screened substances commonly used as cosmetic additives to find substances with antioxidant effects. *Polygonum cuspidatum* (*P. cuspidatum*) extract significantly reduced ROS levels in senescent cells. A novel mechanism was discovered in which *P. cuspidatum* extract reduced ROS, a byproduct of inefficient oxidative phosphorylation (OXPHOS), by increasing OXPHOS efficiency. The reduction in ROS by *P. cuspidatum* extract restored senescence-associated phenotypes and enhanced skin protection. Then, we identified polydatin as the active ingredient of *P. cuspidatum* extract that exhibited antioxidant effects. Polydatin, which contains stilbenoid polyphenols that act as singlet oxygen scavengers through redox reactions, increased OXPHOS efficiency and subsequently restored senescence-associated phenotypes. In summary, our data confirmed the effects of *P. cuspidatum* extract on senescence rejuvenation and skin protection through ROS reduction. This novel finding may be used as a treatment in senescence rejuvenation in clinical and cosmetic fields.

Keywords: reactive oxygen species (ROS); oxidative stress; senescence rejuvenation; *Polygonum cuspidatum*; skin aging



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1. Introduction

The skin is composed of three layers (the epidermis, dermis, and subcutaneous fat) and is an epithelial tissue that covers our body [1]. The skin acts as a barrier to the environment, protects against microorganisms, and maintains fluid and temperature. Skin aging is a process in which skin quality deteriorates with age [2]. Skin aging is indicated by changes in the function of cellular organelles, most notably mitochondrial degeneration [3]. As skin ages, defective mitochondria accumulate and undergo structural changes that significantly increase mitochondrial volume and size [4]. Defective mitochondria leak electrons from complex I and III in the electron transport complex (ETC) to generate ROS as byproducts [5]. In addition to producing ROS, defective mitochondria are the target of oxidative stress, which further increases mitochondrial ROS generation. Increase in oxidative stress by ROS

Article

Assessing the Distribution and Richness of Mammalian Species Using a Stacking Species Distribution Model in a Temperate Forest

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Simple Summary: This study examined the question of how many different mammalian species reside in the Province of Chungnam, Korea, and the influence of environmental, climatic, and human factors. A special model that combines the distribution of each species was used. Distance to forest boundary, elevation, slope, human population density, and distance to water streams were the major factors in determining overall species richness, while climate factors showed relatively less importance. Of particular interest was the fact that the map of unique species aligned with the location of many mammal species. The model would be very helpful to land managers, as well as those who are seeking a method for accommodating greater species diversity.

Abstract: This study was conducted as an effort to examine the association between mammalian species richness and environmental, anthropogenic, and bioclimate factors in the Province of Chungnam, Korea, using a stacked species distribution model (SSDM) approach. An SSDM model was constructed using an extensive dataset collected from 1357 mammal sampling points and their corresponding forest, geographical, anthropogenic, and bioclimatic information. Distance to forest edge, elevation, slope, population density, and distance to water channels were identified as important variables for determining species richness, whereas the impact of bioclimate variables was less important. The endemism map showed a strong correlation with species richness, suggesting the important role of endemic species. Overestimation was observed in areas with lower species richness. However, the findings of the study still demonstrated that valuable insights can be obtained through the use of the SSDM, which may be helpful to land managers, aiding in the effective management of wildlife habitats, particularly in regions with an abundance of species richness and endemism.

Keywords: habitat suitability; species richness mapping; macroecological models; conservation



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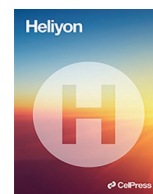
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1. Introduction

In recent decades, species distribution models (SDMs) have been utilized as essential tools for understanding the suitability of current habitats and evaluating biological responses to specific or changing environmental factors [1]. Increased numbers of large datasets with spatial information, incorporating diverse species, and advances in computing capabilities and modeling techniques have led to increased efforts toward the development of SDMs [2,3]. Thus, various SDMs using different modeling/machine learning approaches, including generalized linear models (GLMs), generalized additive models (GAMs), multivariate adaptive regression splines (MARSs), artificial neural networks (ANNs), classification and regression trees (CARTs), maximum entropy (MaxEnt), gradient boosting machine (GBM), and random forest (RF), have been suggested and applied to many regions and taxonomy [4].



Research article

Metabarcoding of pathogenic parasites based on copro-DNA analysis of wild animals in South Korea

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ABSTRACT

Four species of dominant wild animals, namely, *Prionailurus bengalensis euptilurus*, *Nyctereutes procyonoides koreensis*, *Hydropotes inermis argyropus*, and *Sus scrofa coreanus*, are hosts of potential infectious agents, including helminths and protozoa. Therefore, it is necessary to analyze the infectious agents present in these wild animals to monitor and control the spread of pathogens. In the present study, fecal samples from 51 wild animals were collected from the mountains of Yangpyeong, Hoengseong, and Cheongyang in South Korea and metabarcoding of the V9 region of the 18S rRNA gene was performed to identify various parasite species that infect these wild animals. Genes from nematodes, such as *Metastrongylus* sp., *Strongyloides* spp., *Ancylostoma* sp., and *Toxocara* sp., were detected in the fecal samples from wild animals. In addition, platyhelminthes, including *Spirometra* sp., Echinostomatidae gen. sp., *Alaria* sp., *Neodiplostomum* sp., and *Clonorchis* sp., and protozoa, including *Entamoeba* sp., *Blastocystis* sp., *Isospora* sp., *Trichomonas* sp., *Pentatrichomonas* sp., and *Cryptosporidium* sp., were detected. In the present study, various parasites infecting wild animals were successfully identified using metabarcoding. Our technique may play a crucial role in monitoring parasites within wild animals, especially those causing zoonoses.

ASV Amplicon sequence variants
GPS Global positioning system
OTU Operational taxonomic unit
PCR Polymerase chain reaction

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ARTICLE OPEN



Integration of whole-exome sequencing and structural neuroimaging analysis in major depressive disorder: a joint study

Eun-Young Oh^{1,5}, Kyu-Man Han^{2,3,5}, Aram Kim⁴, Youbin Kang⁴, Woo-Suk Tae³, Mi-Ryung Han¹✉ and Byung-Joo Ham¹✉

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Major depressive disorder (MDD) is a common mental illness worldwide and is triggered by an intricate interplay between environmental and genetic factors. Although there are several studies on common variants in MDD, studies on rare variants are relatively limited. In addition, few studies have examined the genetic contributions to neurostructural alterations in MDD using whole-exome sequencing (WES). We performed WES in 367 patients with MDD and 161 healthy controls (HCs) to detect germline and copy number variations in the Korean population. Gene-based rare variants were analyzed to investigate the association between the genes and individuals, followed by neuroimaging-genetic analysis to explore the neural mechanisms underlying the genetic impact in 234 patients with MDD and 135 HCs using diffusion tensor imaging data. We identified 40 MDD-related genes and observed 95 recurrent regions of copy number variations. We also discovered a novel gene, *FRMPD3*, carrying rare variants that influence MDD. In addition, the single nucleotide polymorphism rs771995197 in the *MUC6* gene was significantly associated with the integrity of widespread white matter tracts. Moreover, we identified 918 rare exonic missense variants in genes associated with MDD susceptibility. We postulate that rare variants of *FRMPD3* may contribute significantly to MDD, with a mild penetration effect.

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INTRODUCTION

Major depressive disorder (MDD) is a common psychiatric disorder and has a significant impact on an individual's quality of life, including both social and economic aspects [1]. MDD is characterized by the occurrence of a distinct depressive episode lasting for a minimum of 2 weeks, accompanied by significant changes in mood, interest, and enjoyment [2]. Both genetic and environmental factors play key roles in the etiology of MDD, with estimated genetic inheritance rates up to 25% for a severe recurrent MDD [3]. Common variants account for ~21% of the genetic effects of MDD [4], and rare variants with a moderate penetration effect may contribute to the underlying genetic causes of MDD through additional inheritance mechanisms [5, 6].

Genome-wide association studies (GWASs) have played a crucial role in unraveling the genetic basis of complex diseases and quantitative traits [7]. These studies systematically assess common variants, usually with a minor allele frequency (MAF) > 5% [8]. Despite these findings, much of the genetic contribution to complex traits remains undisclosed, even in diseases where large GWAS meta-analyses have been undertaken [9, 10]. Consequently, many studies have been proposed to elucidate the genetic causes of complex diseases from the perspective of "missing heritability" [11–13]. Recent studies using next-generation sequencing (NGS) suggest that rare variants (MAF < 1%) are associated with complex

diseases [14–16]. Thus, rare variants analyses would be promising to clarify additional disease risks or trait variability.

Although studies have indicated a shared genetic basis between common variants associated with lifetime MDD and depressive symptoms in the general population, it remains unclear whether this association applies to rare variants [17]. Advancement in NGS technology can help identify rare variants [18]. Previous studies have conducted gene-based analyses of rare damaging variants to identify genes related to MDD using the UK Biobank exome dataset [19, 20]. Zhou et al. revealed that genes based on rare variants, including *FOXO1*, *MAPK10*, *DLGAP3*, *ARID5B*, *ASXL2*, and *MED13*, were significantly associated with MDD [19]. Cheng et al. performed a gene-based burden test to identify *OR8B4*, *TRAPPC11*, *SBK3*, and *TNRC6B* between patients with MDD with high polygenic risk scores (PRS) and those with low PRS [20].

The pathophysiology of MDD can be characterized by the dysfunction of brain networks involved in emotional regulation, reward processing, and cognitive controls [21, 22]. White matter tract-based structural connectivity provides a physical and structural basis for these psychopathology-related brain networks [23]. A growing body of evidence has shown microstructural abnormalities in white matter tracts measured by diffusion tensor imaging (DTI) in patients with MDD [24, 25]. For example, a recent study using DTI data from 1305 patients with

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Neuroimaging and epigenetic analysis reveal novel epigenetic loci in major depressive disorder

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Abstract

Background. Epigenetic modifications, such as DNA methylation, contribute to the pathophysiology of major depressive disorder (MDD). This study aimed to identify novel MDD-associated epigenetic loci using DNA methylation profiles and explore the correlations between epigenetic loci and cortical thickness changes in patients with MDD.

Methods. A total of 350 patients with MDD and 161 healthy controls (HCs) were included in the epigenome-wide association studies (EWAS). We analyzed methylation, copy number alteration (CNA), and gene network profiles in the MDD group. A total of 234 patients with MDD and 135 HCs were included in neuroimaging methylation analysis. Pearson's partial correlation analysis was used to estimate the correlation between cortical thickness of brain regions and DNA methylation levels of the loci.

Results. In total, 2018 differentially methylated probes (DMPs) and 351 differentially methylated regions (DMRs) were identified. DMP-related genes were enriched in two networks involved in the central nervous system. In neuroimaging analysis, patients with MDD showed cortical thinning in the prefrontal regions and cortical thickening in several occipital regions. Cortical thickness of the left ventrolateral prefrontal cortex (VLPFC, i.e. pars triangularis) was negatively correlated with eight DMPs associated with six genes (*EML6*, *ZFP64*, *CLSTN3*, *KCNMA1*, *TAOK2*, and *NT5E*).

Conclusion. Through combining DNA methylation and neuroimaging analyses, negative correlations were identified between the cortical thickness of the left VLPFC and DNA methylation levels of eight DMPs. Our findings could improve our understanding of the pathophysiology of MDD.

Introduction

Major depressive disorder (MDD), a common psychiatric disorder with a lifetime prevalence of approximately 16%, severely diminishes individuals' quality of life and limits their functioning in family, work, and social lives (Kessler et al., 2007; Kupfer, Frank, & Phillips, 2012). As the neurobiological etiology of MDD, alterations in monoaminergic neurotransmission, dysfunction of neural circuits involved in emotion and reward processing, disturbances in the hypothalamic–pituitary–adrenal (HPA) axis, and changes in neural–immune interactions have also been suggested (Han & Ham, 2021; Kupfer et al., 2012; Malhi & Mann, 2018). The genetic heritability of MDD is estimated to be approximately 37% (Flint & Kendler, 2014), and genetic makeup can determine the risk of MDD through its genetic influences on the aforementioned neurobiological mechanisms in an interactive manner with psychosocial environmental factors such as childhood maltreatment or stressful life events (Klengel & Binder, 2013).

Regarding the etiology of MDD, gene–environmental interactions suggest that the impacts of psychosocial environmental exposure may be highly dependent on one's genetic vulnerability, which, in turn, can regulate the neurobiological mechanisms of response or coping with stressful stimuli such as early life adversities (Klengel & Binder, 2013). Epigenetic mechanisms such as DNA methylation are deeply involved in the fine modulation of the complex interplay between genes and environments through their pivotal role as mediators of the effects of adverse environmental factors on the genome in MDD (Lopizzo et al., 2015). DNA methylation, particularly methylation changes within promoter and enhancer regions of the gene, has been reported to have long-term effects on the transcription of genes involved in the HPA axis (e.g. *FKBP5*, *NR3C1*) (Efstathiopoulos et al., 2018; Humphreys et al., 2019; Klinger-König et al., 2019), neuroplasticity (e.g. *BDNF*) (Ferrer et al., 2019; Januar, Ancelin, Ritchie, Saffery, &



NRF2 is a spatiotemporal metabolic hub essential for the polyfunctionality of Th2 cells

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Upon encountering allergens, CD4⁺ T cells differentiate into IL-4-producing Th2 cells in lymph nodes, which later transform into polyfunctional Th2 cells producing IL-5 and IL-13 in inflamed tissues. However, the precise mechanism underlying their polyfunctionality remains elusive. In this study, we elucidate the pivotal role of NRF2 in polyfunctional Th2 cells in murine models of allergic asthma and in human Th2 cells. We found that an increase in reactive oxygen species (ROS) in immune cells infiltrating the lungs is necessary for the development of eosinophilic asthma and polyfunctional Th2 cells in vivo. Deletion of the ROS sensor NRF2 specifically in T cells, but not in dendritic cells, significantly abolished eosinophilia and polyfunctional Th2 cells in the airway. Mechanistically, NRF2 intrinsic to T cells is essential for inducing optimal oxidative phosphorylation and glycolysis capacity, thereby driving Th2 cell polyfunctionality independently of IL-33, partially by inducing PPAR γ . Treatment with an NRF2 inhibitor leads to a substantial decrease in polyfunctional Th2 cells and subsequent eosinophilia in mice and a reduction in the production of Th2 cytokines from peripheral blood mononuclear cells in asthmatic patients. These findings highlight the critical role of Nrf2 as a spatial and temporal metabolic hub that is essential for polyfunctional Th2 cells, suggesting potential therapeutic implications for allergic diseases.

NRF2 | metabolic fitness | Th2 cell | OXPHOS | PPAR γ

Unnecessarily aggressive helper T cell immunity is a main cause of chronic immune disorders such as autoimmune or allergic diseases (1, 2). In particular, polyfunctional T cells that can perform multiple functions contribute to the pathogenesis of immune disorders by producing multiple cytokines. For instance, polyfunctional Th17 cells producing high levels of IL-17, IFN γ , and TNF α have been found to contribute to joint inflammation and damage in arthritis (3, 4). In cases of allergic diseases, polyfunctional Th2 cells producing a combination of type 2 cytokines including IL-4, IL-5, and IL-13 are found in the inflamed tissues (5). Polyfunctional T cells are typically generated in response to repeated exposure to antigens in the nonlymphoid tissues, as opposed to initially differentiated effector T cells in the lymph nodes, which typically have less polyfunctionality (6).

The mechanism by which initial effector T cells acquire polyfunctionality remains incompletely understood. One possible mechanism is the cytokine gene cluster that contains several genes encoding cytokines and chemokines. For instance, genes encoding IL-4, IL-5, and IL-13 are clustered within a 150 kb region in the same chromosome, termed “Th2 cytokine gene cluster” (7), suggesting that these type 2 cytokine genes might be regulated in a coordinated manner. Notably, type 2 immune cells including Th2 cells and type 2 innate lymphoid cells (ILC2s) show divergent expression of these cytokines depending on the types of cells and tissues during allergic responses. Th2 cells initially primed in the draining lymph nodes express IL-4 with little IL-5 and IL-13, while Th2 cells in the allergic lungs and skins coexpress IL-5 and/or IL-13 in addition to IL-4 (8–10). Thus, Th2 cells need to receive cues in the nonlymphoid tissues, such as lung and skin, to terminally differentiate into IL-5- and/or IL-13-producing polyfunctional Th2 cells in allergic diseases (2, 11). Tissue-derived alarmin cytokines such as IL-33 have been proposed to be responsible for the differentiation of polyfunctional Th2 cells in the tissues (9). By contrast, several studies have reported that ST2-deficiency failed to reduce, but rather enhance, the production of type 2 cytokines from Th2 cells in animal models (12, 13), suggesting that IL-33 may not be involved in the differentiation of polyfunctional Th2 cells. In this regard, it has been shown that ST2 expression is selectively increased on memory Th2 cells, and thus IL-33 induces the production of IL-5 from memory Th2 cells, but not from effector Th2 cells (14). Considering these findings, it is feasible to surmise that tissue-specific or cell-intrinsic factors other than alarmins

Significance

Initially, differentiated Th2 cells are capable of producing IL-4. These cells then migrate into inflamed tissues, where they further differentiate into polyfunctional Th2 cells capable of producing multiple cytokines including IL-5, IL-9, and IL-13. The molecular mechanism by which initial Th2 cells acquire polyfunctionality in tissues remains unclear. We demonstrate that cell-intrinsic NRF2, a transcription factor responsible for antioxidant responses, is essential for the differentiation of polyfunctional Th2 cells in allergic asthma. Mechanistically, NRF2 drives optimal oxidative phosphorylation (OXPHOS) and glycolysis in Th2 cells, partially through PPAR γ , to establish polyfunctionality in tissue-infiltrating Th2 cells. Since NRF2 also regulates polyfunctionality of human Th2 cells, our findings accentuate the possibility of targeting NRF2–OXPHOS/glycolysis pathway to treat Th2 cell-related diseases in humans.

The authors declare no competing interest.

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RESEARCH ARTICLE



A pan-cancer analysis unveiling the function of NR4A family genes in tumor immune microenvironment, prognosis, and drug response

Seong-Woo Park¹ · Mi-Ryung Han^{1,2}

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Abstract

Background NR4A family genes play crucial roles in cancers. However, the role of NR4A family genes in cancers remains paradoxical as they promote or suppress tumorigenesis.

Objective We aimed to conduct comprehensive analyses of the association between the expression of NR4A family genes and tumor microenvironment (TME) based on bioinformatics methods.

Methods We collected RNA-seq data from 33 cancer types and 20 normal tissue sites from the TCGA and GTEx databases. Expression patterns of NR4A family genes and their associations with DNA methylation, miRNA, overall survival, drug responses, and tumor microenvironment were investigated.

Results Significant downregulation of all NR4A family genes was observed in 15 cancer types. DNA promoter methylation and expression of NR4A family genes were negatively correlated in five cancers. The expression of 10 miRNAs targeting NR4A family genes was negatively correlated with the expression of NR4A family genes. High expression of all NR4A family genes was associated with poor prognosis in stomach adenocarcinoma and increased expressions of *NR4A2* and *NR4A3* were associated with poor prognosis in adrenocortical carcinoma. In addition, we found an elevated expression of *NR4A2*, which enhances the response to various chemotherapeutic drugs, whereas *NR4A3* decreases drug sensitivity. Interestingly, in breast cancer, *NR4A3* was significantly associated with C2 (IFN- γ dominant), C3 (inflammatory), and C6 (TGF- β dominant) immune subtypes and infiltrated immune cell types, implying both oncogenic and tumor-suppressive functions of *NR4A3* in breast cancer.

Conclusion The NR4A family genes have the potential to serve as a diagnostic, prognostic, and immunological marker of human cancers.

Keywords NR4A family genes · Pan-cancer · Bioinformatics · Immune subtype · Tumor microenvironment (TME)

Introduction

Cancer is the leading cause of early mortality in several countries (CP et al. 2020). The incidence and mortality rates of cancer are rapidly increasing worldwide (Sung et al. 2021). There were 10.3 million cancer deaths and

19.3 million new cancer cases in 2020. Thus, it is important to enhance our comprehensive understanding of tumor suppressors and oncogenes.

In previous studies, nuclear receptor subfamily 4 group A (NR4A) family genes were found to be significantly dysregulated in various types of cancers (Mohan et al. 2012). NR4A is a subfamily of transcription factors consisting of three members: *NR4A1*, *NR4A2*, and *NR4A3* (Beard et al. 2015). They have similar domain structures, including N- and C-terminal domains containing activation function domains, and ligand-binding domain (LBD), which surround a DNA-binding domain, and a hinge region (Safe and Karki 2021). These transcription factors respond to various signals and have various biological functions in humans by regulating the pathways involved in homeostasis, proliferation, cell migration, apoptosis, metabolism, DNA repair, and

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RESEARCH

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Integrated analysis of spatial transcriptomics and CT phenotypes for unveiling the novel molecular characteristics of recurrent and non-recurrent high-grade serous ovarian cancer

Hye-Yeon Ju^{1†}, Seo Yeon Youn^{2†}, Jun Kang³, Min Yeop Whang⁴, Youn Jin Choi^{4,5*} and Mi-Ryung Han^{1,6*}

Abstract

Background High-grade serous ovarian cancer (HGSOC), which is known for its heterogeneity, high recurrence rate, and metastasis, is often diagnosed after being dispersed in several sites, with about 80% of patients experiencing recurrence. Despite a better understanding of its metastatic nature, the survival rates of patients with HGSOC remain poor.

Methods Our study utilized spatial transcriptomics (ST) to interpret the tumor microenvironment and computed tomography (CT) to examine spatial characteristics in eight patients with HGSOC divided into recurrent (R) and challenging-to-collect non-recurrent (NR) groups.

Results By integrating ST data with public single-cell RNA sequencing data, bulk RNA sequencing data, and CT data, we identified specific cell population enrichments and differentially expressed genes that correlate with CT phenotypes. Importantly, we elucidated that tumor necrosis factor- α signaling via NF- κ B, oxidative phosphorylation, G2/M checkpoint, E2F targets, and MYC targets served as an indicator of recurrence (poor prognostic markers), and these pathways were significantly enriched in both the R group and certain CT phenotypes. In addition, we identified numerous prognostic markers indicative of nonrecurrence (good prognostic markers). Downregulated expression of *PTGDS* was linked to a higher number of seeding sites (≥ 3) in both internal HGSOC samples and public HGSOC TCIA and TCGA samples. Additionally, lower *PTGDS* expression in the tumor and stromal regions was observed in the R group than in the NR group based on our ST data. Chemotaxis-related markers (*CXCL14* and *NTN4*) and markers associated with immune modulation (*DAPL1* and *RNASE1*) were also found to be good prognostic markers in our ST and radiogenomics analyses.

Conclusions This study demonstrates the potential of radiogenomics, combining CT and ST, for identifying diagnostic and therapeutic targets for HGSOC, marking a step towards personalized medicine.

Keywords High-grade serous ovarian cancer, Spatial transcriptomics, Radiogenomics, Tumor microenvironment

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Transcriptomic analysis of cellular senescence induced by ectopic expression of ATF6α in human breast cancer cells

Ju Won Kim, So-Hyun Bae, Yesol Moon, Eun Kyung Kim, Yongjin Kim, Yun Gyu Park, Mi-Ryung Han, Jeongwon Sohn

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Abstract

Background

The transcriptomic profile of cellular senescence is strongly associated with distinct cell types, the specific stressors triggering senescence, and temporal progression through senescence stages. This implies the potential necessity of conducting separate investigations for each cell type and a stressor inducing senescence. To elucidate the molecular mechanism that drives endoplasmic reticulum (ER) stress-induced cellular senescence in MCF-7 breast cancer cells, with a particular emphasis on the ATF6α branch of the unfolded protein response. We conducted transcriptomic analysis on MCF-7 cells by ectopic expression of ATF6α.

Methods

Transcriptomic sequencing was conducted on MCF-7 cells at 6 and 9 hours post senescence induction through ATF6α ectopic expression. Comprehensive analyses encompassing enriched functional annotation, canonical pathway analysis, gene network analysis, upstream regulator analysis and gene set enrichment analysis were performed on Differentially Expressed Genes (DEGs) at 6 and 9 hours as well as time-related DEGs. Regulators and their targets identified from the upstream regulator analysis were validated through RNA interference, and their impact on cellular senescence was assessed by senescence-associated β-galactosidase staining.

Results

ATF6α ectopic expression resulted in the identification of 12 and 79 DEGs at 6 and 9 hours, respectively, employing criteria of a false discovery rate < 0.05 and a lower fold change (FC) cutoff $|\log_2FC| > 1$. Various analyses highlighted the involvement of the UPR and/or ER Stress Pathway. Upstream regulator analysis of 9 hour-DEGs identified six regulators and eleven target genes associated with processes related to cytoskeleton and 'cell viability and cell death of connective tissue cells.' Validation confirmed the significance of MAP2K1/2, GPAT4, and PDGF-BB among the regulators and DDIT3, PPP1R15A, and IL6 among the targets.

Conclusion

Transcriptomic analyses and validation reveal the importance of the MAP2K1/2/GPAT4-DDIT3 pathway in driving cellular senescence following ATF6α ectopic expression in MCF-7 cells. This study contributes to our understanding of the initial molecular events underlying ER stress-induced cellular senescence in breast cancer cells, providing a foundation for exploring cell type- and stressor-specific responses in cellular senescence induction.

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Data Availability: All relevant data are within the manuscript and its [Supporting Information](#) files. In addition, We have deposited our RNAseq data files in the Gene Expression Omnibus (GEO) database. The data generated in this study is available in the GEO database under accession code GSE270286. The URL for accessing the data is <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE270286>.

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Competing interests: The authors have declared that no competing interests exist.

Abbreviations: ATF6, activating transcription factor 6α; ER, Endoplasmic Reticulum; UPR, unfolded protein response; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TIS, therapy-induced cellular senescence; DEG, differentially expressed genes; siRNA, small interfering RNA; SA-β-gal, senescence associated-β-galactosidase



OPEN **MethylCallR : a comprehensive analysis framework for Illumina Methylation Beadchip**

Hyun-Ho Yang¹ & Mi-Ryung Han^{1,2}✉

DNA methylation is a molecular process that mediates gene-environment interactions. Epigenome-wide association studies (EWAS) using the Illumina Human Methylation BeadChip are powerful tools for quantifying the relationship between DNA methylation and phenotypes. Recently, the Illumina Methylation EPICv2 BeadChip (EPICv2) was released, which includes new features, such as duplicated probes and changed probe names. Several published algorithms have been updated to address these features in EPICv2. However, appropriate EPICv2 preprocessing and integration with previous microarray versions remain complex. Therefore, MethylCallR, an open-source R package designed to provide standard procedures for performing EWAS using Illumina methylation microarrays including EPICv2, was developed. MethylCallR can be used to control duplicated probes in EPICv2, by using pre-set data implemented in MethylCallR or new customized data. MethylCallR includes a straightforward conversion function between different types of Illumina Human Methylation BeadChips. Using MethylCallR, potential outlier sample detection and statistical power estimation were conducted and used to select meaningful probes. Publicly available data was analyzed using MethylCallR and the findings were compared to that of a previous study.

DNA methylation is one of the most well-studied molecular process that is involved in environmental factors and gene-environment interactions^{1,2}. In recent years, significant associations have been identified between variations in DNA methylation and various phenotypes (e.g., aging, body mass index, smoking status, and various diseases)^{3–6}. Epigenome-wide association studies (EWAS) are among the most powerful methods used for statistically assessing the associations between epigenetic variations across the genome and various phenotypes⁷. Microarrays, the most commonly used platform for conducting EWAS, are popular owing to their low resource consumption, high reproducibility, and comprehensive genome coverage⁸. The Illumina Human Methylation BeadChip is the primary platform chosen by researchers since its reliability. Because of its popularity, the Illumina Methylation EPICv2 BeadChip (EPICv2) has recently been released as a successor to the Illumina Methylation EPIC BeadChip (EPICv1) and Illumina Methylation 450 K BeadChip (450 K). With the powerful advantages of microarrays and improved performance, EPICv2 is expected to become the primary choice for researchers planning to analyze DNA methylation^{9–11}.

Unlike EPICv1 and 450 K, EPICv2 requires several new feature considerations for analysis: (i) exactly duplicated probes, (ii) duplicated probes in the probe sequence, (iii) duplicated probes in position, and (iv) probes with different names compared to previous versions. Controlling duplicate probes is important when interpreting results or performing meta-analyses using data from EPICv1 or 450 K. Although detailed descriptions and functional testing of duplicated probes have been reported by Peters et al. fully controlling the replicated probes remains a challenge¹⁰. In particular, the reliance on external reference datasets for analysis, such as reference-based cell composition estimation, has become more complicated^{12,13}. Considering that the available algorithms are primarily optimized for EPICv1, it is important to apply them to EPICv2.

To date, well-structured popular pipelines have been widely used to conduct EWAS, which are designed to facilitate the easy utilization of numerous algorithms^{14–18}. Meffil is an EWAS pipeline that includes solutions for efficient analysis of large-scale DNA methylation data¹⁸. DMRcate algorithm utilizes Gaussian kernel smoothing to identify significantly differentially methylated regions (DMRs)¹⁹. Conumee2.0 is an algorithm that includes useful functions for identifying significant copy number variations, such as tangent normalization, circular binary segmentation, and segmented block bootstrapping²⁰. Although these algorithms have recently been updated to be compatible with EPICv2, only a few EWAS pipelines support the analysis of EPICv2.

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RESEARCH

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Epigenetic regulation on left atrial function and disease recurrence after catheter ablation in atrial fibrillation

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Abstract

Background Genetic variation and modifiable risk factors play a significant role in the pathogenesis of atrial fibrillation (AF). The influence of epigenetic modification on AF remains to be elucidated. We investigated the role of DNA methylation in the etiology of AF. Epigenetic evaluation was performed in 115 AF patients who underwent radiofrequency catheter ablation in a single institution. We measured methylation at approximately 850,000 bp cytosine-phosphate-guanine (CpG) sites in the 115 samples. The degree of methylation was compared across seven classification criteria: type of AF, late recurrence, impaired left atrium (LA) function, late gadolinium enhancement, LA diameter, LA volume, and flow velocity of the LA appendage.

Results The four most significantly methylated genes were *DEFB104B*, *C3*, *TANC1*, and *TMEM9B*. The *DEFB104B* gene (cg20223677 in the transcription start site), which encodes β -defensin 104B, was hypomethylated in three groups: AF patients with late recurrence, impaired LA function, and impaired LAA flow velocity. Enriched functional annotation of the differentially methylated datasets revealed that five out of the seven AF groups in this cohort were associated with genes involved in the cell movement of endothelial cell lines, sprouting angiogenesis by endothelial cell lines, or migration of endothelial cell lines.

Conclusions Epigenetic profiling revealed that epigenetic modification might affect important characteristics of AF. Our results suggest that the pathogenesis of AF might be affected by not only genetic variation or modifiable factors but also by epigenetic modulation.

Keywords Atrial fibrillation, Epigenetics, Catheter ablation, Recurrence

Introduction

Atrial fibrillation (AF) is associated with significant impairment in quality of life and various major adverse cardiovascular events. Due to the aging of the general population, the incidence and global medical burden of AF is rising rapidly [1]. Treatment of AF requires understanding of its pathophysiology. Clinical risk factors for AF include age, sex, lifestyle habits, and cardiovascular comorbidities [2, 3]. Genetic predisposition is another important risk factor for the development of AF. Previous studies identified several genetic variations that are potentially responsible for the occurrence of AF [4–6]. In

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The Effect of Forkhead Box O1 Single Nucleotide Polymorphisms on Cortical Thickness and White Matter Integrity in High Suicide Risk Patients

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Objective Neuroinflammation's role is increasingly emphasized in the pathology of major depressive disorder (MDD), and its close association with the risk of suicide is being reported. The Forkhead Box O1 (FoxO1) gene is known to play a role in regulating mood and emotion and is associated with susceptibility to suicidality in relation to environmental stress. This research aims to explore the relationship between FoxO1 and the risk of suicide in individuals with MDD.

Methods We enrolled 127 healthy controls (HC) and 231 patients diagnosed with MDD, including 119 individuals with high suicide risk (HSR). All participants underwent the Hamilton Rating Scale for Depression Assessment and magnetic resonance imaging. Cortical thickness and white matter integrity were evaluated.

Results In the HSR group, cortical thinning was observed in the left triangular part of the inferior frontal gyrus and right transverse frontopolar gyrus compared to HC. Additionally, fractional anisotropy (FA) values were decreased in the left posterior thalamic radiation, sagittal stratum, and uncinate fasciculus. Although no differences were observed based on allele variations for the two FoxO1 single nucleotide polymorphisms (SNPs), those with the minor allele of FoxO1 rs34733279, especially in the HSR group, displayed increased cortical thinning and reduced FA values in the left cingulum.

Conclusion Our study reveals close association between the minor allele of the FoxO1 gene rs34733279 and suicide risk in the left cingulum highlights the potential key role of the FoxO1 gene rs34733279 in the context of suicidal vulnerability. Further investigations are warranted to elucidate the underlying biological mechanisms.

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Keywords Forkhead box protein O1; Single nucleotide polymorphisms; Suicide risk; Cortical thickness; Tract-based spatial statistics.

INTRODUCTION

Suicide is a severe social issue with a global age-standardized suicide rate of 10.5 per 100,000 in 2016, as per the World Health Organization report.¹ Suicide is the second most common cause of premature death among those aged 15–29,

following traffic accidents, and the third most common cause among those aged 15–44.² Suicide leads to economic and emotional burdens for bereaved families.^{3–6} And major depressive disorder (MDD) is the leading cause of suicide, with a 20-fold increase in suicide risk in individuals with MDD, and >50% of suicide deaths being linked to mood disorders.^{7,8} Although the mechanisms underlying depression have been extensively studied, it is crucial to explore the biological mechanisms underlying suicide risk.

Previous studies have shed light on serotonergic mechanism abnormalities, including elevated serotonin receptor subtypes and reduced serotonin metabolites.^{9–11} Moreover, elevated neuroinflammation, indicated by increased levels of inflammatory markers such as interleukin-6 and other cytokines in the bloodstream or cerebrospinal fluid, has been de-

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Rice *OsGATA16* is a positive regulator for chlorophyll biosynthesis and chloroplast development

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SUMMARY

Chloroplasts are essential organelles in plants that contain chlorophylls and facilitate photosynthesis for growth and development. As photosynthetic efficiency significantly impacts crop productivity, understanding the regulatory mechanisms of chloroplast development has been crucial in increasing grain and biomass production. This study demonstrates the involvement of *OsGATA16*, an ortholog of Arabidopsis *GATA*, *NITRATE INDUCIBLE*, *CARBON-METABOLISM INVOLVED* (*GNC*), and *GNC-LIKE/CYTOKININ-RESPONSIVE GATA FACTOR 1* (*GNL/CGA1*), in chlorophyll biosynthesis and chloroplast development in rice (*Oryza sativa*). The *osgata16-1* knockdown mutants produced pale-green leaves, while *OsGATA16*-overexpressed plants (*OsGATA16*-OE1) generated dark-green leaves, compared to their parental *japonica* rice. Reverse transcription and quantitative PCR analysis revealed downregulation of genes related to chloroplast division, chlorophyll biosynthesis, and photosynthesis in the leaves of *osgata16-1* and upregulation in those of *OsGATA16*-OE1. Additionally, *in vivo* binding assays showed that *OsGATA16* directly binds to the promoter regions of *OsHEMA*, *OsCHLH*, *OsPORA*, *OsPORB*, and *OsFtsZ*, and upregulates their expression. These findings indicate that *OsGATA16* serves as a positive regulator controlling chlorophyll biosynthesis and chloroplast development in rice.

Keywords: rice, *OsGATA16*, chlorophyll biosynthesis, chloroplast development, *OsHEMA*, *OsCHLH*, *OsPORA*, *OsPORB*, *OsFtsZ*.

INTRODUCTION

Phototrophic organisms utilize chloroplasts to convert light energy into chemical energy, such as ATP and NADPH, which is necessary for plant growth (Barber et al., 2000; Grossman et al., 1995). Chlorophylls (Chls) are the pigments responsible for harvesting light energy within the chloroplasts. Colorless proplastids have the potential to differentiate into chloroplasts in response to various environmental and developmental cues, including light and phytohormone cytokinin (Jarvis & López-Juez, 2013; Waters & Langdale, 2009).

The synthesis of chlorophylls in land plants, green algae, and cyanobacteria involves the tetrapyrrole biosynthesis pathway (Jarvis & López-Juez, 2013; Martin et al., 2002; Waters & Langdale, 2009). The biosynthesis of chlorophyll begins with the conversion of glutamate, which

leads to the formation of protoporphyrin-IX (Proto) (Brzezowski et al., 2015; Larkin, 2016). The Mg-chelatase complex, encoded by *GUN5/CHLH* (*GENOMES UNCOUPLED5*/Mg-chelatase subunit H), *CHLD*, and *CHLI1* or *CHLI2*, catalyzes the insertion of Mg²⁺ into Proto, resulting in the formation of Mg-Proto. This is subsequently converted into protochlorophyllide *a* (Pchlide *a*) (Brzezowski et al., 2015; Larkin, 2016), which is then converted to chlorophyllide *a* (Chlide *a*) via the action of protochlorophyllide oxidoreductases (PORs) in a light-dependent manner (Kwon et al., 2017; Masuda & Takamiya, 2004; Sakuraba et al., 2013). The POR is a nuclear-encoded single polypeptide, and its isoforms are highly conserved in plant species. For example, the Arabidopsis genome encodes three PORs (PORA, PORB, and PORC) (Oosawa et al., 2000). Barley, rice, and tobacco genomes contain two PORs, while



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journal homepage: www.keaipublishing.com/en/journals/the-crop-journal/Rice *ONAC016* promotes leaf senescence through abscisic acid signaling pathway involving *OsNAP*Eunji Gi^{a,1}, Sung-Hwan Cho^{a,1}, Suk-Hwan Kim^a, Kiyoon Kang^{b,*}, Nam-Chon Paek^{a,*}^a Department of Agriculture, Forestry and Bioresources, Plant Genomics and Breeding Institute, Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea^b Division of Life Sciences, Incheon National University, Incheon 22012, Republic of Korea

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ABSTRACT

Senescence-induced NAC (senNAC) TFs play a crucial role in senescence during the final stage of leaf development. In this study, we identified a rice senNAC, *ONAC016*, which functions as a positive regulator of leaf senescence. The expression of *ONAC016* increased rapidly in rice leaves during the progression of dark-induced and natural senescence. The *onac016-1* knockout mutant showed a delayed leaf yellowing phenotype, whereas the overexpression of *ONAC016* accelerated leaf senescence. Notably, *ONAC016* expression was upregulated by abscisic acid (ABA), and thus detached leaves of the *onac016-1* mutant remained green much longer under ABA treatment. Quantitative RT-PCR analysis showed that *ONAC016* upregulates the genes associated with chlorophyll degradation, senescence, and ABA signaling. Yeast one-hybrid and dual-luciferase assays revealed that *ONAC016* binds directly to the promoter regions of *OsNAP*, a key gene involved in chlorophyll degradation and ABA-induced senescence. Taken together, these results suggest that *ONAC016* plays an important role in promoting leaf senescence through the ABA signaling pathway involving *OsNAP*.

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1. Introduction

The process of leaf senescence is accompanied by extensive degradation of macromolecules and dramatic changes in gene expression [1]. The onset and progression of leaf senescence are finely tuned by a combination of endogenous signals mediated by phytohormones and external factors including drought, high salinity, light, nutrient starvation, and pathogen attack [1–4].

One of the best approaches to understanding the mechanisms of leaf senescence is the isolation and analysis of mutants that exhibit delayed leaf senescence [5]. To date, several genes have been identified and characterized as regulators of leaf senescence [6,7]. In *Arabidopsis*, the transcription of more than 40 senescence-associated transcription factors (senTFs) increases during senescence [8]. These senTFs include the plant-specific NAM/ATF1/ATF2/CUC2 (NAC) TFs, which are named as senescence-induced NACs (senNACs) [8]. For instance, *ORESARA1* (*ORE1/ANAC092*) and *AtNAP* (*ANAC029*) are positive regulators of leaf senescence [9,10]. Mutation of *ORE1* and *AtNAP* delays leaf senescence, whereas over-

expression of *ORE1* and *AtNAP* promotes leaf senescence. Contrary to the positive regulation of *ORE1* and *AtNAP* on leaf senescence, *JUNGBRUNNEN1* (*JUB1/ANAC042*) and *VNDINTERACTING2* (*ANAC083/VNI2*) were identified as negative regulator of leaf senescence. Transgenic *Arabidopsis* plants overexpressing *JUB1* and *VNI2* delayed leaf senescence [11,12].

Among 151 rice (*Oryza sativa*) NAC genes (*OsNACs*) [13], several *OsNACs* were identified as *senNACs*. Especially, *OsNAP* (*Os03g21060*) is a functional ortholog of *AtNAP* [10]. *OsNAP* directly upregulates chlorophyll degradation genes (CDGs) such as *STAY-GREEN* (*SGR*), *Red chlorophyll catabolite reductase 1* (*RCCR1*), *NYC1*, and *NYC3*, and senescence-associated genes (SAGs) such as *Osh36*, *Osl57*, *Osh69*, and *Osl85*, thereby promoting the leaf senescence under both natural senescence (NS) and dark-induced senescence (DIS) [14]. In contrast to the upregulation of *OsNAP* in the expression of CDGs and SAGs, *ONAC106* negatively regulates their expressions [15]. Thus, the rice plants overexpressing *ONAC106* maintain chlorophyll levels much longer and delay leaf senescence during NS and DIS.

Phytohormones act as signaling molecules and regulators of various cellular processes, including leaf senescence and abiotic stress responses. Among them, abscisic acid (ABA) is a key phytohormone that mediates plant responses to environmental stress

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Rice CHD3/Mi-2 chromatin remodeling factor RFS regulates vascular development and root formation by modulating the transcription of auxin-related genes *NAL1* and *OsPIN1*

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The vascular system in plants facilitates long-distance transportation of water and nutrients through the xylem and phloem, while also providing mechanical support for vertical growth. Although many genes that regulate vascular development in rice have been identified, the mechanism by which epigenetic regulators control vascular development remains unclear. This study found that Rolled Fine Striped (RFS), a Chromodomain Helicase DNA-binding 3 (CHD3)/Mi-2 subfamily protein, regulates vascular development in rice by affecting the initiation and development of primordia. The *rfs* mutant was found to affect auxin-related genes, as revealed by RNA sequencing and reverse transcription-quantitative PCR analysis. The transcript levels of *OsPIN1* and *NAL1* genes were downregulated in *rfs* mutant, compared to the wild-type plant. The chromatin immunoprecipitation assays showed lower levels of H3K4me3 in the *OsPIN1a* and *NAL1* genes in *rfs* mutant. Furthermore, exogenous auxin treatment partially rescued the reduced adventitious root vascular development in *rfs* mutant. Subsequently, exogenous treatments with auxin or an auxin-transport inhibitor revealed that the expression of *OsPIN1a* and *NAL1* is mainly affected by auxin. These results provide strong evidence that *RFS* plays an important role in vascular development and root formation through the auxin signaling pathway in rice. [BMB Reports 2024; 57(10): 441-446]

INTRODUCTION

Vascular bundles of plants, composed of xylem and phloem tissues, connect all parts of the plant body from leaf to root through their conductive function, providing water and nutrients transport, mechanical support, and overall structural strength (1). Auxin, which is important for vascular establishment and maintenance, is mainly synthesized in the plant cells of actively growing tissues, such as developing leaves and roots, and transported to other tissues to participate in organ primordia formation, apical meristem maintenance, and vascular tissue differentiation through the process of polar auxin transport (2-4).

Auxin flow distribution and gradient establishment are regulated by the specific cellular auxin transport proteins (5). PIN proteins are known to determine the direction and distribution of auxin movement (5, 6). In *Arabidopsis*, five PINs are predominantly localized in the plasma membrane, and determine the direction of intracellular auxin flux to maintain auxin gradient distribution (7). The *Arabidopsis pin* mutants show growth defects in shoot and stems, root, inflorescence, and embryo (3, 8). The rice genome contains twelve PIN genes (9). The *ospin* mutants are reported to be involved in root development, plant height, tiller and panicle branch angle, panicle formation, yield, and tiller growth (10, 11). Several mutants have been reported to affect rice vascular development and root formation by regulating the expression of *OsPIN* genes. For example, the mutation of *NARROW LEAF1* (*NAL1*) caused defective cell division by affecting polar auxin transport *OsPINs*, and showed reduction in veins and adventitious root formation (12, 13). The *NARROW LEAF2/3* (*NAL2/3*) plays a key regulatory role in lateral axis outgrowth, vascular development, and root formation, through the regulation of *OsPIN* genes (14-16).

Several studies have reported the key role of chromodomain helicase DNA-binding (CHD) family proteins as transcriptional regulators in the regulation of developmental processes and stress response (17, 18). In *Arabidopsis*, PICKLE belongs to typical CHD protein, while the *pkl* mutant shows pleiotropic defects, such as semi-dwarfism and reduced root meristem acti-

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ORIGINAL ARTICLE



Rice CRYPTOCHROME-INTERACTING BASIC HELIX-LOOP-HELIX 1-LIKE interacts with OsCRY2 and promotes flowering by upregulating *Early heading date 1*

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Abstract

Flowering time is a crucial adaptive response to seasonal variation in plants and is regulated by environmental cues such as photoperiod and temperature. In this study, we demonstrated the regulatory function of rice *CRYPTOCHROME-INTERACTING BASIC HELIX-LOOP-HELIX 1-LIKE* (*OsCIBL1*) in flowering time. Overexpression of *OsCIBL1* promoted flowering, whereas the *oscib1l* knockout mutation did not alter flowering time independent of photoperiodic conditions. Cryptochromes (CRYs) are blue light photoreceptors that enable plants to sense photoperiodic changes. *OsCIBL1* interacted with *OsCRY2*, a member of the rice CRY family (*OsCRY1a*, *OsCRY1b*, and *OsCRY2*), and bound to the *Early heading date 1* (*Ehd1*) promoter, activating the rice-specific *Ehd1-Heading date 3a/RICE FLOWERING LOCUS T 1* pathway for flowering induction. Dual-luciferase reporter assays showed that the *OsCIBL1*-*OsCRY2* complex required blue light to induce *Ehd1* transcription. Natural alleles resulting from nonsynonymous single nucleotide polymorphisms in *OsCIBL1* and *OsCRY2* may contribute to the adaptive expansion of rice cultivation areas. These results expand our understanding of the molecular mechanisms controlling rice flowering and highlight the importance of blue light-responsive genes in the geographic distribution of rice.

KEYWORDS

flowering time, light-responsive genes, natural variation

1 | INTRODUCTION

Flowering, an essential process for plant reproduction and survival, is influenced by both internal genetic factors and external cues such as photoperiod and temperature. Understanding flowering mechanisms is essential for successful plant reproduction in a world facing climate change. Changes in flowering time caused by fluctuations in temperature

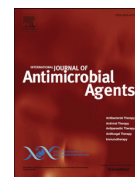
and day length can threaten the yield of food crops that support more than half of the world's population (Craufurd & Wheeler, 2009; Tun et al., 2021). A thorough understanding of flowering regulation is crucial for achieving agricultural success and protecting food security as the climate becomes increasingly unpredictable.

Photoperiod is a critical environmental cue that allows plants to perceive and adapt to environmental changes. Flowering plants are

Sang-Ji Lee, Yunjeong Kim, and Kiyoon Kang contributed equally to this work.

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Short Communication

Boosting with variant-matched adenovirus-based vaccines promotes neutralizing antibody responses against SARS-CoV-2 Omicron sublineages in mice

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ABSTRACT

Global spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) Omicron subvariants, such as BA.4, BA.5 and XBB.1.5, has been leading the recent wave of coronavirus disease 2019 (COVID-19). Unique mutations in the spike proteins of these emerging Omicron subvariants caused immune evasion from the pre-existing protective immunity induced by vaccination or natural infection. Previously, we developed AdCLD-CoV19-1, a non-replicating recombinant adenoviral vector that encodes the receptor binding domain of the spike protein of the ancestral SARS-CoV-2 strain. Based on the same recombinant adenoviral vector platform, updated vaccines that cover unique mutations found in each Omicron subvariant, including BA.1, BA.2, BA.4.1 and BA.5, were constructed. Preclinical studies revealed that each updated vaccine as a booster shot following primary vaccination targeting the ancestral strain improved neutralizing antibody responses against the pseudovirus of its respective strain most effectively. Of note, boosting with a vaccine targeting the BA.1 or BA.2 Omicron subvariant was most effective in neutralization against the pseudovirus of the BA.2.75 strain, whereas BA.4.1/5-adapted booster shots were most effective in neutralization against the BQ.1, BQ.1.1 and BF.7 strains. Therefore, it is imperative to develop a vaccination strategy that can cover the unique spike mutations of currently circulating Omicron subvariants in order to prevent the next wave of COVID-19.

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1. Introduction

The recent surge in coronavirus disease 2019 (COVID-19) driven by the global spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) Omicron variants, such as BA.4, BA.5, BQ.1 and XBB.1.5, presents a new challenge for the application of current COVID-19 vaccines and therapeutics [1–4]. The latest studies have shown that sera from individuals vaccinated with primary mRNA vaccine series followed by boosting with the original vac-

cines, or vaccinated individuals who experienced breakthrough infection with the BA.1 subvariant of the Omicron lineage have a diminished neutralizing capacity against the BA.4 and BA.5 strains compared with the BA.1 and BA.2 strains, presumably due to the L452R and F486V mutations in the receptor-binding domain of the spike protein [1,2,5]. Another Omicron subvariant, BA.2.75, has nine unique mutations in the spike protein compared with the BA.2 strain [6,7]. While the BA.2.75 strain showed no significant antibody evasion against sera collected after the BA.1/2 wave, its descendant, the BN.1 strain, was increased in frequency in Australia and the UK, which may be due to three additional spike mutations (K356T, R346T and F490S) compared with the BA.2.75 strain [6,8–10]. Additionally, the emergence of new BA.5-derived Omicron subvariants, including the BQ.1 and XBB.1.5 strains, is contributing to

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Review Article



IL-17 and IL-21: Their Immunobiology and Therapeutic Potentials

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ABSTRACT

Studies over the last 2 decades have identified IL-17 and IL-21 as key cytokines in the modulation of a wide range of immune responses. IL-17 serves as a critical defender against bacterial and fungal pathogens, while maintaining symbiotic relationships with commensal microbiota. However, alterations in its levels can lead to chronic inflammation and autoimmunity. IL-21, on the other hand, bridges the adaptive and innate immune responses, and its imbalance is implicated in autoimmune diseases and cancer, highlighting its important role in both health and disease. Delving into the intricacies of these cytokines not only opens new avenues for understanding the immune system, but also promises innovative advances in the development of therapeutic strategies for numerous diseases. In this review, we will discuss an updated view of the immunobiology and therapeutic potential of IL-17 and IL-21.

Keywords: Interleukin-21; Interleukin-17; Autoimmune; Cancer; Inflammation

INTRODUCTION

Cytokines are essential components of the body that determine the nature and magnitude of immune responses. They facilitate cellular interactions that enable a sophisticated and effective immune response to various biological challenges, such as inflammation and cancer (1). By influencing cell growth, differentiation, movement, and inflammatory responses, they play a critical role in maintaining the body's equilibrium and enhancing its defenses against pathogens. However, alterations in their activities can lead to various pathological conditions, from inflammatory and autoimmune diseases to increased susceptibility to infection, highlighting their complex role in health and disease (2).

IL-17 and IL-21 are key players in the body's immunological orchestra, each providing unique and vital elements to the overall functionality of our defense mechanisms—host defense immunity. IL-17 is a versatile cytokine that is essential for shaping inflammatory responses and acting as the first line of defense against bacterial and fungal invasion (3). It's key in mediating inflammation and maintaining the integrity of the body's barriers against environmental and microbial threats. Derived from diverse cells such as Th17 cells, $\gamma\delta$ T



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The role of alternative autophagy in cell viability and response to paclitaxel treatment in v-Ha-ras-transformed NIH 3T3 cells

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ABSTRACT

In confluent v-Ha-ras-transformed NIH 3T3 fibroblasts (Ras-NIH 3T3), LC3 downregulation may precede a decrease in canonical autophagy, thus contributing to cell survival. Herein, we aimed to investigate the role of alternative autophagy in the viability of long-term cultures of Ras-NIH 3T3 cells and their parental NIH 3T3 cells. As cell confluence increased with the culture period, the level of alternative autophagy, as assessed through Lamp2-Rab9 co-localization, gradually decreased in both cell lines. However, Ras-NIH 3T3 cells maintained higher levels of alternative autophagy than the parental cells did. Rab9 knockdown minimally affected NIH 3T3 cells while drastically reducing the viability of Ras-NIH 3T3 cells, which suggested that alternative autophagy plays a critical role in Ras-NIH 3T3 cells. In contrast, reactive oxygen species (ROS) production in Ras-NIH 3T3 cells was higher than that in NIH 3T3 cells during long-term culture. Moreover, NIH 3T3 cells exhibited a continual decrease in mitochondrial mass, whereas Ras-NIH 3T3 cells maintained high mitochondrial mass. Immunofluorescence analysis of mitochondrial membrane marker proteins and mitochondrial membrane potential (MMP) also demonstrated a temporal pattern of changes similar to those of mitochondrial mass. This finding could be attributed to the relatively higher level of alternative autophagy in Ras-NIH 3T3 cells facilitating the removal of damaged mitochondria. Paclitaxel treatment in Ras-NIH 3T3 cells induced an increase in canonical autophagy rates along with suppression of alternative autophagy. Ras-NIH 3T3 cells showed high sensitivity to paclitaxel at the early stage of culture, but as cell confluence increased, resistance to paclitaxel increased, showing a similar level of cell viability to the vehicle control group. The study findings suggest that alternative autophagy is more important than canonical autophagy for maintaining cell survival in response to an unfavorable environment, such as during high cell confluence and exposure to anticancer agents.

1. Introduction

Noncanonical alternative autophagy is a novel pathway activated in response to metabolic stress in cells lacking Atg5/LC3-dependent canonical autophagy [1]. While the membranes used for autophagosome formation during canonical autophagy originate from the endoplasmic reticulum and outer mitochondrial membranes, those utilized in alternative autophagy originate from the Golgi membranes [2]. Canonical autophagy is implicated in the early stage tumorigenesis inhibition and subsequent progression [3,4]. However, the role of the recently identified pathway of alternative autophagy in this context remains unclear. Understanding the relationship between alternative autophagy and tumorigenesis may thus help develop novel treatments for cancer.

Rab9 is a small GTPase that plays a vital role in transporting diverse molecules for recycling or degradation within the endosome [5]. It plays an essential role in autophagosome formation during alternative autophagy [6]. Notably, recent study has highlighted the upregulation of Rab9 in various cancers, including breast, prostate, liver, and pancreatic cancers [7]. Moreover, elevated Rab9 expression has been linked to an unfavorable patient prognosis and reduced overall survival rates [8]. Although the exact mechanisms through which Rab9 contributes to tumorigenesis remain unclear, it has been proposed to participate in the transport of signaling molecules that are involved in the invasion and metastasis of cancer cells [9]. Rab9-dependent autophagy has also been linked to the regulation of mitochondrial function [10].

We recently reported that v-Ha-ras-transformed NIH 3T3 cells (Ras-

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Review

Anti-Cancer Strategy Based on Changes in the Role of Autophagy Depending on the Survival Environment and Tumorigenesis Stages

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Abstract: Autophagy is a crucial mechanism for recycling intracellular materials, and under normal metabolic conditions, it is maintained at low levels in cells. However, when nutrients are deficient or under hypoxic conditions, the level of autophagy significantly increases. Particularly in cancer cells, which grow more rapidly than normal cells and tend to grow in a three-dimensional manner, cells inside the cell mass often face limited oxygen supply, leading to inherently higher levels of autophagy. Therefore, the initial development of anticancer drugs targeting autophagy was based on a strategy to suppress these high levels of autophagy. However, anticancer drugs that inhibit autophagy have not shown promising results in clinical trials, as it has been revealed that autophagy does not always play a role that favors cancer cell survival. Hence, this review aims to suggest anticancer strategies based on the changes in the role of autophagy according to survival conditions and tumorigenesis stage.

Keywords: canonical autophagy; alternative autophagy; cellular transformation; tumor; microenvironment; anticancer drugs



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1. Introduction

Autophagy is a cellular process in which intracellular materials are delivered to the lysosome for degradation [1], and refers to the concept that cells can survive through the recycling of materials under nutrient-deprived conditions. Yoshinori Ohsumi, awarded the Nobel Prize in Physiology or Medicine in 2016 for his discovery of autophagy mechanisms, identified several autophagy-related genes that are known as APG through yeast genetic screening [2]. Since autophagy plays a crucial role in degrading unnecessary proteins and cellular organelles, its dysregulation is associated with several diseases, including neurodegenerative disorders [3–5], as well as malignant tumors such as those of the liver, colon, stomach, and breast [6]. In particular, there has been significant research on the role of autophagy in tumorigenesis, with many studies reporting the development of liver tumors in autophagy-deficient mouse models [7–9]. Our laboratory has also confirmed that the loss of *ATG5* induces cellular transformation [10].

It has been reported that a type of autophagy called alternative autophagy exists, which shares similarities with canonical autophagy but operates independently of *ATG5*/*ATG7* [11,12]. Although the exact role and mechanism of alternative autophagy remain unclear, there is a key difference in the membrane source. Canonical autophagy originates from the endoplasmic reticulum (ER) and mitochondria-associated ER membranes, whereas alternative autophagy originates from the trans-Golgi membrane [11]. In addition, in canonical autophagy, *ATG5*, *ATG7*, and *LC3* are involved in autophagosome formation. However, in alternative autophagy, the Rab9 protein, which participates in late endosome-trans-Golgi transport, attaches to the autophagic membrane, leading to the generation of autophagosomes and autolysosomes [13,14]. Studies have shown that alternative autophagy plays an

Review of the genus *Xyrosaris* Meyrick (Lepidoptera, Yponomeutidae, Yponomeutinae) from Korea, with two new species

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Abstract

The genus *Xyrosaris* Meyrick, 1907 from Korea is reviewed. Two new species, *Xyrosaris vaginata* **sp. nov.** and *X. triacantha* **sp. nov.** are described from Korea, along with notes on allied species of the genus. In addition, *X. insularis* Ponomarenko & Beljaev, 2023 is reported for the first time from Korea, including a new host plant. Key to species, illustrations of adult, male and female genitalia, diagnoses, host plants, and descriptions are provided.

Key words: distribution, Korea, new species, taxonomy

Introduction

The genus *Xyrosaris* Meyrick, 1907, which belongs to the subfamily Yponomeutinae of the family Yponomeutidae, is distributed throughout the world. The genus was established based on the type species, *X. dryopa* Meyrick, 1907. A total of thirteen species were recognized in the world (Lewis & Sohn 2015; Agassiz 2019; Bippus 2020; Ponomarenko & Beljaev 2023).

In the Australasian region, *X. dryopa* Meyrick and *X. acroxutha* Turner were reported in Australia. In the Oriental region, *X. maligna* Meyrick, *X. campsiptila* Meyrick, and *X. lirinopa* Meyrick were recorded in India, Sri Lanka, and China. In the Ethiopian region, *X. secreta* Meyrick, *X. obtorta* Meyrick, *X. lucae* Agassiz, *X. louisae* Agassiz, and *X. canusa* Bippus have been reported in the Afrotropical and Madagascan area. In the Neotropical region, only one species, *X. mnesicentra* Meyrick was recorded. In the East Asia, *X. lichneuta* Meyrick and *X. insularis* Ponomarenko & Beljaev have been reported up to date (Lewis & Sohn 2015; KSAE & ESK 2021; Bippus 2020; Ponomarenko & Beljaev 2023).

In the present study, we describe two new species, *Xyrosaris vaginata* **sp. n.** and *X. triacantha* **sp. n.**, from Korea, along with notes on allied species, *X. lichneuta* Meyrick and *X. insularis* Ponomarenko & Beljaev 2023. Key to species, illustrations of adult, male and female genitalia, diagnoses, host plants, and description are provided.

Materials and methods

A majority of species examined is based on specimens collected from Korea. The specimens were collected at the lights with a mercury vapor lamp (220V/ 200W, Dongseong Co., Korea) and four UV lamps (20W, FL20SbL/18, Kumho Co., Korea). Genitalia were dissected and examined under a Leica EZ4 stereo-microscope (Leica microsystems, CMS GmbH, Wetzlar, Germany). Images of the adults and genitalia were taken with a Leica M165C (Leica microsystems, Wetzlar, Germany) attached to a Dhyana 400DC CMOS camera (Tucsen photonics, China), and a Carl Zeiss, DE/Axio Imager M2 (Carl Zeiss Microscopy, GmbH, Königsallee, Göttingen, Germany). The

New findings of poronotic oribatid mites (Acari: Oribatida) from Korea

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Abstract

This work deals with 13 species of oribatid mites from different regions of Korea. Two new species, *Humerobates aokii* **sp. nov.** and *Humerobates ulleungdoensis* **sp. nov.** are proposed, and 11 known species, *Punctoribates ezoensis* (Fujikawa, 1982), *Tectoribates proximus* (Berlese, 1910), *Protoribates tohokuensis* Fujikawa, 2003, *Protoribates capucinus* Berlese, 1908, *Peloribates pilosus* Hammer, 1952, *Scheloribates fimbriatus* Thor, 1930, *Neoribates aurantiacus* (Oudemans, 1914), *Zygoribatula glabra* (Michael, 1890), *Pergalumna myrmophila* (Berlese, 1914), *Trichogalumna boninensis* Hagino, Bayartogtokh & Shimano, 2017, and *Trichogalumna ohkuboi* Hagino, Bayartogtokh & Shimano, 2017 are newly reported for the fauna of Korea. Supplementary descriptions and illustrations of each species along with their distributional data are provided.

Key words: Brachypylina, Poronota, *Humerobates*, soil mites, new species, new record, Korea

Introduction

Porose areas and its related organs are one of the important characters in systematics of oribatid mites, and about half of all known species being poronotic. There are several types of porose integumental organs in oribatid mites, and their structures and functions might be different (Alberti *et al.* 1997; Norton *et al.* 1997). The cuticular part is called a porose area if it is not invaginated. If invaginated, porose areas have a variety of names, *i.e.*, small pouches are saccules; large, flat, lamelliform pouches are platytracheae; thick, relatively short tubes are brachytracheae; and extremely long, filamentous tubes are tubules (Behan-Pelletier & Lindo, 2023).

There are 24 superfamilies in Brachypylina, which is the largest, most taxonomically rich group of oribatid mites, and seven superfamilies (Licneremaeoidea, Phenopeloipoidea, Achipterioidea, Oribatelloidea, Oripodoidea, Ceratozetoidea, and Galumnoidea) are often considered a natural, monophyletic subgroup based on their possession of the octotaxic system in adults (Norton & Behan-Pelletier, 2009).

When dermal glands occur on the notogaster of Brachypylina, they are typically arranged in four pairs, forming the octotaxic system of porose organs. In rare cases, the octotaxic system may include a mixture of porose areas and saccules. While the typical complement is four pairs of octotaxic organs, higher or lower numbers are not uncommon (Norton & Alberti 1997; Behan-Pelletier 2015).

This work is a part of our ongoing studies on oribatid mites of Korea, and is concerned on species belonging to the families Humeroatidae Grandjean, 1971, Punctoribatidae Thor, 1937, Tectoribatidae Grandjean, 1954, Haplozetidae Grandjean, 1936, Scheloribatidae Grandjean, 1933, Oribatulidae Thor, 1929, Parakalumidae Grandjean, 1936, collected from the central and southern regions of the country.

We dedicate this work in memory of our esteemed teacher and colleague, Dr. Jun-ichi Aoki, who was a world leading specialist for oribatid mites. He had exceptional abilities and experiences in discovering soil mites, and was one of the scientists who led to the development of modern acarology in Japan, Korea, China, Mongolia, Thailand and some other countries. He was a pioneer in soil zoological research in Korea studying soil mites, and published the first taxonomic works (Aoki 1966, 1974, 1975).

Three new species of *Cirrothaumatia* Razowski & Becker from Peru, with notes on the genus (Lepidoptera: Tortricidae: Tortricinae: Cochylini)

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Abstract

Three new species of the cochyline genus *Cirrothaumatia* Razowski & Becker, 1986 are described and illustrated from submontane Andean sites in Peru (Depts. Amazonas, Junín, and San Martín): *C. pichita* n. sp., *C. huemboana* n. sp., and *C. moyobamba* n. sp. Comments and illustrations are provided for the three previously described species from southern Mexico (Veracruz), Central America (Guatemala, Costa Rica, and Panama), and northern South America (Venezuela: Edo. Aragua; Ecuador: Pichincha Prov.).

Key words: Andean, Central America, Costa Rica, distribution, Ecuador, Guatemala, Mexico, Neotropical, Panama, Peru, South America, taxonomy, Venezuela

Introduction

Cirrothaumatia Razowski & Becker, 1986, was previously known from three species distributed in submontane habitats from southern Mexico (Veracruz) to western Panama in Central America, and in South America at one site in northern Venezuela (Edo. Aragua) and one site in northern Ecuador (Pichincha) (Razowski and Pelz 2001). We discovered three new species from Peru that indicate that the genus is a complex of closely related species, with the new species considerably disjunct from the known distributions of the other *Cirrothaumatia* species. The three new species represent the first records from the central Andes (eastern foothills, to 2165 m elevation), considerably southward from the nearest species in Venezuela in the northernmost Andean spur-range of northern Venezuela, along the Caribbean Coast of Edo. Aragua (1100 m) (see map Fig. 18). A single specimen from Ecuador identified by Razowski and Pelz (2001) as *C. tornosema* (Clarke, 1968) requires further study to confirm whether it is conspecific with a described species from Central America or perhaps another undescribed species.

Cirrothaumatia species appear to be inhabitants of submontane forests, ranging in elevations from 600–1100 m in Costa Rica, 800 m in western Panama, 1100 m in northern Ecuador, 1100 m in northern Venezuela, and 1300 m in southern Mexico (Veracruz), with one of the new sites in Peru the highest known thus far for the genus—2165 m (Fig. 10). The other two sites in Peru are at 970 m and 2085 m, each with a different new species. Based on male genitalia, Razowski and Becker (1986) considered *Cirrothaumatia* to be related to *Parirazona* Razowski 1984c. The new species from Peru are each based on a single female specimen, but clearly have genital and adult features very similar to the other known *Cirrothaumatia* species (Figs. 7–9). The discovery of the new species is somewhat of a surprise since the cochylini of Central and South America have been reviewed in a series of papers

Taxonomic study of the genus *Spilonota* Stephens (Lepidoptera: Tortricidae: Olethreutinae: Eucosmini) with descriptions of two new species in Korea

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Abstract

A taxonomic study of *Spilonota* Stephens, 1834, in Korea is conducted, and *S. prognathana* (Snellen, 1883) which had previously been merged with *S. albicana* (Motschulsky, 1866) is separated again. Additionally, as a result of research based on materials from Incheon National University two new species; *S. samseong* Choi, Bae & Nasu, *S. laticucullusa* Choi, Bae & Nasu proposed from Korea. The study provides brief descriptions of *Spilonota* species in Korea, with illustrations of the adult and genital morphology. Identification key for the known species reported from Korea is included.

Key words: Tortricoidea, distribution, taxonomy, host plant, new species

Introduction

Spilonota Stephens (1834) was established with type species *Tortrix ocellana* ([Denis & Schiffermüller], 1775), and it belongs to the tribe Eucosmini, subfamily Olethreutinae. The genus comprises 41 recorded species worldwide (Gilligan *et al.* 2018), which distributed in the Holarctic region, including Korea, China, Japan, Russia, Southeast Asia, India, Europe, North America and Australian region. The larvae of *Spilonota* species feed on leaves, stems, and roots, and they include leaf rollers, leaf webbers, and leaf miners (Roelofs & Brown 1982). *Spilonota* larvae include some of the notorious fruit pests that feed mainly on leaves, but occasionally attack fruit (Park *et al.* 1977).

In Korea, three species of *Spilonota* have been recorded (Zoological Society of Korea 1968): *S. prognathana* (Snellen, 1883), *S. lechriaspis* Meyrick, 1932 and *S. ocellana* ([Denis & Schiffermüller], 1775). After that, it was reorganized by Park (1983). *S. prognathana* was synonymised with *S. albicana*, resulting in the addition of one unrecorded species, *S. eremitana* Moriuti, 1972. Byun & Park (1993) added one for the unrecorded species, *S. semirufana* (Christoph, 1882).

In this study, we investigated deposited specimens in the collections of Incheon National University in order to clarify the species of *Spilonota* in Korea. According to Park (1983), *S. prognathana* is a synonym of *S. albicana*. However, through the study records of Kuznetsov (2001), Oku (2003) and Nasu (2021), *S. prognathana* and *S. albicana* are separated again. In addition, we provide brief descriptions of Korean *Spilonota*, including two new species. Additionally, a taxonomic key and illustrations of adults and genitalia of all Korean species are provided.

Review of the genus *Rhagoba* Moore, 1888 (Lepidoptera, Crambidae, Spilomelinae) from Laos, with a description of new species

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Abstract

The genus *Rhagoba* from Laos is revised, with three species, *R. octomaculalis* (Moore, 1867), *R. obvellata* Du & Li, 2012, and *R. flavolineata* **sp. nov.** is reported from Laos for the first time. A key to the Laotian species of *Rhagoba* is provided, with illustrations of adults and genitalia of examined species.

Key words: Margaroniini, *Pygospila*, new record, taxonomy, Oriental Region

Introduction

The genus *Rhagoba* Moore, 1888 is a member of the tribe Margaroniini (Mally *et al.* 2019), and it was erected for the type species, *Filodes octomaculalis* Moore, 1867, from Darjeeling, India. *Rhagoba* currently consists of two oriental species worldwide: *R. octomaculalis* (Moore, 1867) and *R. obvellata* Du & Li, 2012 (Nuss *et al.* 2003–2024; Du & Li 2012).

In Laos, only a single species, *R. octomaculalis* (Moore, 1867), of this genus was recorded by Kitajima (2020). In this study, we reviewed *Rhagoba* from Laos, including a new species, *R. flavolineata* **sp. nov.** and a newly recorded species, *R. obvellata*. Additionally, we provided a key to the species of *Rhagoba*, along with illustrations of adults and genitalia.

Materials and Methods

The specimens used in this study were collected during expeditions in Laos under the Memorandum of Understanding (MOU) with the National Institute of Biological Resources (NIBR), Ministry of Environment, Korea, and the Department of Forest Resources Management, Ministry of Natural Resources and Environment, Laos, for cooperation concerning biological resources and information. Korean specimens used in this study are deposited in the collection of Incheon National University, Korea (INU). Types of the new species and all examined specimens are deposited in the following collections: Incheon National University (INU) and the National Institute of Biological Resources (NIBR), Ministry of Environment, Korea. The specimens were collected using light sheets with a high-pressure mercury vapor lamp (450W) and four black light lamps (FL20SbL).



Original Article

An illustrated checklist of the tribe Nomophilini (Lepidoptera: Spilomelinae) with a newly recorded genus in Korea

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ABSTRACT

The current knowledge of the tribe Nomophilini in Korea is reported, based on several taxa described to date. In addition, we report *Syngamia falsidicalis* (Walker, 1859) and the genus *Syngamia* Guenée (1854) for the first time in Korea. A total of 10 species, in 6 genera, are verified for Korea. Illustrations of adults and genitalia, and a key to genera of Nomophilini are provided.

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Introduction

Kuznetsov and Stekolnikov (1979) established the tribe Nomophilini, based on the genus *Nomophila* Hübner, 1825. A total of 359 described species of 25 genera belonging to this tribe are known in the world (Nuss et al. 2003–2023). Recently, two molecular phylogenetic studies were conducted for Spilomelinae, resulting in the division of Nomophilini into two clades by Mally et al. (2019) and Matsui et al. (2022). In Korea, the first record of Nomophilini species was published by Leech and South (1901), with two species as the subfamily Pyraustinae. To date, seven species of four genera within Nomophilini were reported in Korea (Yanamaka and Sasaki 2013; Bae 2021; Nuss et al. 2003–2023). From the late 1920s, several Nomophilini species in Korea were reported by Maruta (1929), the Zoological Society of Korea (1968), Pak (1970), Park et al. (2008), and Shin et al. (2022).

In this study, we include a checklist of 10 species in 6 genera of Nomophilini in Korea. The genus *Syngamia* Guenée (1854) and the

species *S. falsidicalis* (Walker, 1859) are recorded for the first time in Korea. Additionally, we follow the combination of Heppner and Inoue (1992) for *Bocchoris aptalis* (Walker, 1866) to the genus *Sameodes*. All taxa are arranged alphabetically, with illustrations of adults and genitalia of Nomophilini species.

Material and methods

The specimens used in this study are deposited in the collection of Incheon National University (INU), Hannam University (HNU), National Institute of Biological Resources (NIBR), Korea National Arboretum (KNA), and National Institute of Agricultural Sciences (NAS), South Korea. Images of adults and genitalia were taken using a Leica S6D (Wetzlar, Germany) stereomicroscope attached to a Dhyana 400DC (Tucsen Photonics, Fuzhou, China), with a dome illuminator Leica LED5000 HDI (Wetzlar, Germany). Genitalia were dissected and examined under a Leica EZ4 stereomicroscope (Wetzlar, Germany). Abbreviations used are as follows: TS (type species); TL (type locality); GG (Gyeonggi-do); GW (Gangwon-do); CB (Chungcheongbuk-do); JB (Jeollabuk-do); JN (Jeollanam-do); GB (Gyeongsangbuk-do); GN (Gyeongsangnam-do); JJ (Jeju-do).

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New and little-known species of soil mites of the families Oppiidae and Suctobelbidae (Acari: Oribatida) from Korea

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ABSTRACT

Both families studied here, Oppiidae and Suctobelbidae, are diversely represented in the northern hemisphere, but these groups are insufficiently studied in Korea. Herein, we propose two new species, *Ramusella (Insculptoppiella) ermilovi* **sp. nov.**, *Medioxyoppia jejudoensis* **sp. nov.**, and provide supplementary descriptions of 11 little known species, *Graptoppia (Stenoppia) crista* Ohkubo, 1996, *Multioppia glabra* (Mihelčič, 1955), *Ramusella curtipilus* Hammer, 1971, *Quadroppia (Coronoquadroppia) monstrosa* Hammer, 1979, *Suctobelbella amurica* (Krivolutsky, 1966), *Suctobelbella granifera* Chinone, 2003, *Suctobelbella nitida* Chinone, 2003, *Suctobelbella (Flagrosuctobelba) aokii* Chinone, 2003, *Suctobelbella (Flagrosuctobelba) chabarica* Rjabinin, 1975, *Suctobelbella (Ussuribata) chinonei* Subías, 2017, and *Suctobelbella (Ussuribata) variosetosa* (Hammer, 1961), which are newly reported from Korea. All species are inhabitants of litter and soil from various types of forests in Korea.

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Introduction

Both the Oppiidae Sellnick, 1937 and Suctobelbidae Jacot, 1938 are among the largest families of oribatid mites in species richness as well as in distributional range, occurring in nearly all terrestrial habitats worldwide as well as some arboreal and semiaquatic environments. Representatives of these families are widespread, living in all biogeographical regions, and although there are a great number of tropical species, Oppiidae and Suctobelbidae are also very diverse in temperate regions (Subías 2004, 2022).

Their small body size (body length often ~ 160 or more), high diversity, and relatively simple structure make the systematics of Oppiidae as well as Suctobelbidae difficult and complicated. Therefore, both families are still insufficiently known in terms of species diversity in most regions of the world, including Korea, where only 21 and 16 species have been recorded, respectively (Paik 1980, 1985; Choi 1986, 1987, 1988, 1996, 1997, 1998, 1999; Choi and Jung 1998; Bayartogtokh and Bae 2022a, 2022b). These species numbers may be far less than one quarter of the potential species richness of these families in Korea. Therefore, it is highly desirable to discover more unknown species, and at the same time, to redefine the known species more precisely in order to promote the systematics of both Oppiidae and Suctobelbidae.

Moreover, many species have been poorly diagnosed and illustrated with incomplete descriptions, and consequently, these highly variable groups of mites became rather rich with generic and species synonymies, which also causes further problems in ecological or biogeographical investigations. Therefore, redefinition of known taxa is essential for resolving not only species taxonomy, but also supraspecific systematics of these highly diverse groups of oribatid mites.

In the course of our studies on oribatid mites from Korea, we found two new species belonging to the genera *Ramusella* and *Medioxyoppia*, and 11 known species from the genera *Graptoppia*, *Multioppia*, *Quadroppia*, and *Suctobelbella*, which are reported for the first time from Korea. The main aim of the present work is to describe

the new species and to provide supplementary descriptions on the morphology of the little-known species with detailed illustrations.

Materials and methods

The present material was collected from the central and southern parts of Korea, which contain mountainous areas with various types of forests. The collection locality and habitat data for each species are given in the respective “material examined” section.

Mites were extracted from samples by means of modified Berlese funnel extraction. All type specimens as well as other voucher specimens studied here are deposited in the collection of the National Institute of Biological Resources, Incheon, Korea.

Specimens were mounted in lactic acid on temporary cavity slides for measurement and illustration. The body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the ventral plate. Notogastral width refers to the maximum width in dorsal aspect. Lengths of body setae were measured in lateral aspect. Setae were measured in lateral view in which they could be best observed. All measurements are given in micrometres (µm). Formulas for leg setation are given in parentheses according to the sequence trochanter–femur–genu–tibia–tarsus (famulus included). Formulas for leg solenidia are given according to the sequence genu–tibia–tarsus.

Drawings were made with a camera lucida attached to the transmission light microscope Leica DM 2500. Images were obtained with an Tucsen Dhyana 400DC camera using a transmission light microscope Leica DM 2500.

The morphological terminology used in this paper follows that of F. Grandjean: see Travé and Vachon (1975) for references, Norton and Behan-Pelletier (2009), and applied by Bayartogtokh and Bae (2022a, 2022b).

Results

This work deals with six species of Oppiidae and seven species of Suctobelbidae, of which two are represented as new to science,

First report of the family Attevidae (Lepidoptera, Yponomeutoidea) from Cambodia, with description of a new species

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Abstract

In the present study, we report a pantropical family Attevidae of the superfamily Yponomeutoidea in Cambodia for the first time. A new species, *Atteva chalco* **sp. nov.**, and two newly recorded species, *A. fabriciella* Swederus, 1787, *A. wallengreni* Sohn and Wu, 2013 are reported. Illustrations of adults, male and female genitalia, diagnoses, and descriptions are provided.

Key words: Korea, taxonomy, new species, distribution

Introduction

The family Attevidae Mosher, 1916, which belongs to the superfamily Yponomeutoidea is distributed in pantropical areas, exclusive of *Atteva aurea* (Fitch, 1856) that extends to the temperate zone of North America. A total of 48 species were recognized in the world. In the Oriental Region, 19 species of *Atteva* are reported. Still, especially in Mainland Southeast Asia, it was poorly known with 7 species: *Atteva emisella* Walker, *A. fabriciella* Swederus, *A. niveigutta* Walker, *A. pulchella* Moore, *A. subaurata* Durrant, *A. wallengreni* Sohn & Wu, and *A. yanguifella* Sohn & Wu (Sohn & Wu 2013; Lewis & Sohn 2015). Sohn & Wu (2013) reviewed Chinese Attevidae including revised identity of *Atteva fabriciella* Swederus, and designated lectotype of *A. brucea* Moore & *A. niviguttella* Berg. Nevertheless, taxonomic studies on the family Attevidae have never been conducted in Cambodia to date.

In the present study, we report the family Attevidae from Cambodia for the first time with a new species, *Atteva chalco* **sp. nov.**, and two newly recorded species, *A. fabriciella* Swederus, 1787 and *A. wallengreni* Sohn and Wu, 2013. Illustrations of adults, male and female genitalia, diagnoses, and descriptions are provided.

Materials and methods

A majority of species examined is based on specimens collected from Cambodia. The specimens were collected at the lights with a mercury vapor lamp (220V/ 200W, Dongseong Co., Korea) and four UV lamps (20W, FL20SbL/18, Kumho Co., Korea). Genitalia were dissected and examined under a Leica EZ4 stereo-microscope (Leica microsystems, CMS GmbH, Wetzlar, Germany). Images of adults and genitalia were taken with a Leica M165C (Leica microsystems, Wetzlar, Germany) attached to a Dhyana 400DC CMOS camera (Tucsen photonics, China). The examined specimens are deposited in Incheon National University, Incheon, Korea.

Abbreviations used herein are as follows: INUB—Bio-resource and Environmental Center, Department of Life Sciences, Incheon National University; TL—type locality; TS—type species.



Original Article

Two newly recorded species with a revised checklist for the Korean nolid moth fauna (Lepidoptera, Nolidae)



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ABSTRACT

A total of 73 species belonging to 30 genera of the family Nolidae are recognized in Korea. This study reports two additional Nolidae species, with a newly recorded genus *Nanola* László, Ronkay G, Witt, 2010. Additionally, a checklist and illustrations of adults and genitalia of newly recorded species are provided.

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Introduction

Korean nolid moth fauna has been changed by several researchers. Several Korean researchers contributed to the Korean fauna, but more noticeable taxonomic changes were carried out by foreign researchers especially European before 1900's (Fixsen 1887; Staudinger 1887; Leech 1888, 1889, 1900; Hampson 1894). In 1900's Japanese researcher start to contributed Korean fauna, and Korean researcher appeared 1950's (Seitz 1912; Maruda 1929; Matsumura 1930; Doi 1938; Bryk 1948; Pak 1959, 1964; Paik and Park 1963; Inoue 1976, 1982; Park and Lee 1977; Shin 1981, 1984, 1985, 1989; Kim et al. 1982; Poole 1989; Sugi 1990; Ahn et al. 1994; The Entomological Society of Korea and Korean Society of Applied Entomology 1994; Jeong 1997; Kononenko et al. 1998; Kim 1999).

In the new Century, after 2000's, several Korean and foreign researchers newly reported and contributed in Korean Nolidae fauna up to now (Shin 2001; Sohn et al. 2005a, 2005b; Choi 2006, 2011; Kononenko and Han 2007; Tshistjakov 2008; Paek et al. 2010; Sasaki and Kishida 2011; Heo 2012; László et al. 2015; Beljaev et al. 2016; Beljaev and Velyaev 2016; Heo 2016; Kim et al. 2016; Cha et al. 2019, 2020, 2022, 2023; National Institute of Biological Resources 2019, 2022; Bayarsaikhan et al. 2020; Volynkin and Saldaitis 2022). Especially, Zahirri et al. (2013) suggest a new phylogenetic tree of the Nolidae based on molecular and morphological studies. And then, Volynkin and Saldaitis (2022) newly proposed the genus *Agrisius* Walker has been transferred within the Nolidae.

These rapid taxonomic changes make several erroneous about Korean Nolidae. So in this study, the author adopts very recent pieces of information and provides a revised checklist with two newly recorded species: *Manoba melancholica* (Wileman and West 1928) and *Nanola liaoningensis* (Han and Li 2008) from Korea. Among them, the genus *Nanola* László, Ronkay G, Witt, 2010 is firstly recorded in South Korea.

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Original Article

Three newly recorded *Nola* species in Laos (Lepidoptera, Nolidae, Nolinae)[☆]Yeong-Bin Cha^a, Ulziiargal Bayarsaikhan^b, Sora Kim^{a,c}, Yang-Seop Bae^{d,e,*}^a Laboratory of Insect Phylogenetics & Evolution, Department of Plant Protection and Quarantine, Jeonbuk National University, Jeonju, 54896, South Korea^b Convergence Research Center for Insect Vectors, Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University, Incheon, 22012, South Korea^c Department of Agricultural Convergence Technology, Jeonbuk National University, Jeonju, 54896, South Korea^d Bio-Resource and Environmental Center, Incheon National University, Incheon, 22012, South Korea^e Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University, Incheon, 22012, South Korea

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ABSTRACT

The paper contains the diagnosis of *Nola erythrostigmata* species-group of Laotian species. Color figures of adults and genitalia of the examined species are provided.

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Introduction

This paper contained second series of Laotian nolid species collected by Incheon National University, funded by National Institute of Biological Resources (NIBR), during 2015 to 2019. The examined Nolidae specimens for the purposes of this paper have been deposited with NIBR. In Laos, only two *Nola* species (*N. euryzonata* (Hampson) and *N. lucidalis* (Walker)) have been recorded by Cha et al. (2019). This poor research history can be sure of numerous undescribed and unrecorded species.

In this study, *Nola erythrostigmata* species-complex group species are newly recorded in Laos. This species group is comprised four described species, *N. erythrostigmata* Hampson, 1894, *N. danii* László, Ronkay & Ronkay, 2014, *N. lucidalis* (Hampson, 1914), and *N. gyurii* László, Ronkay & Ronkay, 2014 (László et al. 2014), and characterized by the pale ground color of forewing with fuscous or

reddish patch or fascia on medial and distal part (László et al. 2014). Among them, three species (except *N. lucidalis*) are newly recorded. Illustrations of adults and genitalia of all examined species are provided, with synonymic list and distributions.

Material and methods

The examined specimens with genitalia slides are housed in National Institute of Biological Resources, Incheon, Korea. The specimens were captured using vertical light sheets illuminated by a mercury vapor lamp (220V/200W; Dongseong Co., Korea) and four blacklight lamps (20W, FL29SbL/18; Kumho Co., Korea). Genitalia were dissected and examined under a Leica EZ4 stereomicroscope. Images of adults were taken by a Tucsen Dhyana 400DC digital camera attached to a Leica S6D stereomicroscope with dome illuminator Leica LED5000 HDI. Genitalia photograph were taken using a Tucsen Dhyana 400DC digital camera mounted on a Leica S8APO stereomicroscope.

Abbreviations of institute: INU = Incheon National University, Incheon, South Korea; NIBR = National Institute of Biological Resources, Incheon, Korea; NHMUK = British Museum of Natural History, London, United Kingdom; MWM = Museum Witt München, Munich, Germany; RNH = Royal Museum of Natural History (Naturalis Biodiversity Center), Leiden.

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Contribution to the knowledge of soil mites of the family Brachychthoniidae (Acari: Oribatida) in Korea, with key to known species

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Abstract

This work deals with four species of oribatid mites of the family Brachychthoniidae Thor, 1934 from Korea. A new species, *Sellnickochthonius gotjawaliensis* sp. nov. is proposed, and three known species, *Liochthonius leptaleus* Moritz, 1976, *Liochthonius muscorum* Forsslund, 1964, and *Sellnickochthonius cricoides* (Weis-Fogh, 1948) are newly reported to the fauna of Korea. Diagnostic characterization and illustration of each species along with their habitat ecology and distributional data are given. A diagnostic key to adults of all species of Brachychthoniidae known from Korea is presented.

Key words: *Liochthonius*, *Sellnickochthonius*, oribatid mites, new species, new record, Korea

Introduction

Among the early-derivative oribatid mite infraorder Enarthronota, the family Brachychthoniidae Thor, 1934 is with 13 genera (including one subgenus) and about 170 species, one of the largest families in terms of taxonomic diversity (Subías 2004, online update 2024). The family has a worldwide distribution, and its members are often abundant components of the soil, moss, litter, and lichen fauna (Subías *et al.* 2012).

As the representatives of the early-derivative oribatid mites, the adults of Brachychthoniidae share some primitive morphological characters, such as dichoid body shape (with two transverse scissures on the notogaster), 16 pairs of notogastral setae and a peranal segment (Grandjean 1953). The general characters of the family are outlined by Niedbala (1972a, 1973), Chinone and Aoki (1972), Moritz (1976a, b), Balogh and Mahunka (1983), Weigmann (2006), Norton and Behan-Pelletier (2009). The descriptions of the most species of Brachychthoniidae have been exclusively based on adults, and therefore, at the moment, the ontogenetic morphologies of only nine species from five genera are known at different levels (Norton & Ermilov 2014, 2024), of which the complete morphological ontogeny of only three species is known (Grandjean 1963; Seniczak & Seniczak 2017).

This family has been insufficiently studied in Korea, where 18 species belonging to the genera *Brachychthonius* (one sp.), *Eobrachychthonius* (one sp.), *Liochthonius* (eight spp.), *Neobrachychthonius* (one sp.), *Poecilochthonius* (two spp.), *Sellnickochthonius* (four spp.), *Synchthonius* (one sp.) have been recorded (Choi 1997; Bayartogtokh & Bae 2023).

In the course of the faunistic surveys of oribatid mites of Korea, we found one unknown species belonging to the genus *Sellnickochthonius* along with three known species, *Liochthonius leptaleus*

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